Polymorphism of goat DGAT1 gene and their association with milk production traits

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ABSTRACT

The purpose of the study was to identify genotype frequencies of single nucleotide polymorphisms the intron 6, exon 7, intron 7, exon 8, intron 8 and exon 15, intron 15, exon 16, intron 16, exon 17 and partially 3' untranslated region (UTR) in goat DGAT1 gene and its possible association genotypes with milk traits in dairy goat breeds by means of PCR-RFLP assays. Goats 185: Saanen n: 35; Damascus n: 30; Halep n: 30; Maltase n: 30; Alpine n: 30; Kilis n: 30) were used for investigating. PCR products were digested separately with EcoRI, NlaIII and AluI restriction enzymes for exon 8, exon 16 and 17, respectively. No polymorphisms were detected at the EcoRI and AluI cleavage sites for exon 8 and exon 17 in sampled goat populations. However, biallelic polymorphism was found with restriction endonuclease NlaIII in intron 16 of DGAT1 gene and two genotypes were detected and TC, in which TT was the predominant genotype and allele T was predominant allele in six goat breeds. No significant statistical results were founded in milk yield, fat, protein and lactose values with TT and TC genotypes were detected. We have described here for the first time an NlaIII PCR-RFLP method for detecting T-to-C mutation in intron 16 goat DGAT1 locus: CATG-to-CACG.

Key words: DGAT1 gene, Polymorphism, Goat, Milk traits

Milk traits are polygenic and all the genes affecting them are difficult to know but a few potential candidate genes have been recognized (An et al. 2011). Detection of candidate genes and suitable genetic markers for milk production is very important. Polymorphisms within selected candidate genes can be tested for their association with quantitative traits to better understand their effects and can be used in marker-assisted selection (Wu et al. 2005).

Milk fat contains approximately 98% triglycerides (TGs) (Marshall et al. 1997). The final and the only committed step in the biosynthesis of TGs is catalysed by acyl- CoA: diacylglycerol acyltransferase (DGAT) enzymes. The genes encoding two DGAT enzymes, DGAT1 and DGAT2, were identified, and enzyme DGAT1 acts as a catalyst to triacylglycerol synthesis (Yen et al. 2008). DGAT1 is a candidate gene for milk production traits in bovine and other dairy ruminants. Many association studies were applied the DGAT1 gene as a marker for milk yield and milk fat and protein percentages in cattle (Grisart et al. 2002, Winter et al. 2002, Furbass et al. 2006, Spelman et al. 2002) these investigation results showed that polymorphisms in exon 8 of the DGAT1 gene in Bos taurus, AA-GC exchange resulting in a non conservative substitution of amino acid 232 Lysine (allele-K) to Alanine (allele-A). Allele K is a wild type and responsible to increases fat yield of milk and fat and protein content, whereas allele A increases both milk and protein yield in cattle (Grisart et al. 2002, Winter et al. 2002).

Only a few studies (Angiolillo et al. 2007, Miltiadou et al. 2010, Sharma et al. 2011) has been published to date compared with bovine DGAT1 gene polymorphisms associated with milk production in goat breeds. Angiolillo et al. (2007) characterized complete coding region (exon 1–17) of caprine DGAT1 gene, according to their results polymorphisms of the goat DGAT1 gene was found limited. However, a single nucleotide polymorphism (SNP) was reported by them, this SNP was a T to C transition located at intron 16 (Angiolillo et al. 2007).

Kilis, a goat breed in Turkey, has resistance hot and cold weathers and infectious diseases. Its milk is preferred reared in Turkey (Akcapinar 2000). The present study was aimed to identify genotype frequencies of single nucleotide polymorphisms the intron 6, exon 7, intron 7, exon 8, intron 8 and exon 15, intron 15, exon 16, intron 16, exon 17 and partially 3' untranslated region (UTR) in goat DGAT1 gene and its possible association genotypes with milk traits in Saanen, Maltase, Alpine, Damascus, Halep and Kilis dairy goat breeds by means of PCR-RFLP assays.
MATERIALS AND METHODS

Animal resources and DNA isolation: Animals were chosen at random and were 4-year-old, multiparous and lactating. During the day animals grazed on pasture and they received as supplement 250g/head/day concentrate commercial food (crude protein 20% and 2500 ME kcal/kg), animals raised in semi-intensive conditions. Monthly individual milk yield was recorded from 30–day of lactation until 280, 240, 230, 240, 210 day Saanen, Maltase, Alpine, Damascus, Halep and Kilis goat breeds respectively. Animals were milked once a day and 20 ml milk sample was collected for milk analysis. Milk recording was carried out by veterinarians and individual milk yield had been recorded each fifteen a day during lactation and for each individual milk sample were analyzed for fat, protein and lactose using milk analyser.

Jugular blood samples (2 ml/ewe) were collected from 35 Saanen, 30 Damascus, 30 Halep, 30 Kilis, 30 Alpine and 30 Malta goat breeds using EDTA as an anticoagulant. Genomic DNA was extracted from the whole blood using the phenol chloroform method (Sambrook and Russell 2011).

The study was approved by the Ethical Committee of Laboratory Animals, Firat University.

PCR amplification: For this study, according to the goat (DQ380249) sequence of DGAT1 gene, 3 pairs of primers were designed to amplify for intron 6, exon 7, intron 7, exon 8, intron 8 and exon 15, intron 15, exon 16, intron 16, exon 17 and partially 3′ untranslared region (UTR) in DGAT1 gene. Primer pairs and their annealing temperatures are shown in Table 1. For primer design, Primer3 software (Rozen and Skaltsky 2000) was used. PCR reaction was carried out for each primer in 50 μL of total volume, containing 10 X PCR buffer (50 mM/L KCl, 10 mM/L Tris-HCl (pH 8.0), 0.1% Triton X–100), 1.5 mM MgCl2, 0.2 mM of each dNTP, 10 pM/L of each primer, 50 ng ovine genomic DNA and 1U Taq DNA polymerase. PCR conditions were as follows: denaturation at 94 °C for 4 min, followed by 34 cycles of denaturation at 94 °C for 1 min, annealing at X °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 10 min. The PCR products were separated by electrophoresis on 4% agarose gel stained with ethidium bromide. The PCR products showing different band patterns on RFLP gel were selected for sequencing.

Statistical analysis: Direct counting was used to estimate phenotype and allele frequencies of DGAT1 gene EaeI, NlaIII, AluI genetic variants. The Chi-Square Test (χ²) was used to analyse the Hardy-Weinberg equilibrium was performed by PopGene32 software (Yeh et al 2000) (data not shown). ANOVA (one-way) was used to analyses association of DGAT1 variants with milk yield, fat and protein contents using to Minitab statistical software.

RESULTS AND DISCUSSION

The amplicons exon 8 including partial exon 5, whole intron 6, exon 7, intron 7, exon 8 and intron 8, amplicons exon 16 including whole exon 15, intron 15, exon 16, intron 16 and partial exon 17 and amplicons exon 17 including whole intron 15, exon 16, intron 16, exon 17 and partial 3′UTR. The results showed that amplification fragment size (514 bp, 401 bp and 429 bp for exon 8, exon 16 and exon 17 respectively) were consistent with the target one and had good specificity, which could be directly analysed by RFLP assay.

No SNPs were detected in exon 8 and all goat breeds were homozygous for alleles (allele K) encoding Lysine. For exon 17 two AluI cleavage sites (372 and 57 bp) within the DGAT1 gene, but none of these was polymorphic. Allele T, in which the polymorphic restriction sites at 1139 nt position of the DQ380250 is absent, is characterized by the presence of the one fragment of 429 bp length (genotype CC) and sample populations, only one sample shown TC genotype (372 and 57 bp), which is belong to Halep goat breeds (Fig. 3).

The results of RFLP assay presence of six cleavage sites (21–24, 88–91, 194–197, 221–224 and 297–300) within exon 16 - exon 16 sequence, but only one (297–300) was shown to be polymorphic. The DGAT1 intron 16 was shown polymorphism in the Saanen, Maltase, Damascus, Halep and Kilis populations. This SNP was a T to C transition.

### Table 1. Primers used for PCR amplification of the exon 8, exon 16 and exon 17 regions in goat DGAT1 gene

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Annealing temperature (°C)</th>
<th>Amplicon size (bp)</th>
<th>Covered region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 8</td>
<td>CCACCTTGTCTCTG CGCGAAGAGGAA GGTAGTAGAG 53</td>
<td>514</td>
<td>Partial exon 5, Intron6, Exon 7, Intron 7, Exon 8, Intron 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 16</td>
<td>CCCAGACATCTCT TGGCCGAGATGTA GTGACAG 54</td>
<td>401</td>
<td>Exon 15, Intron 15, Exon 16, Intron 16, partial exon 17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 17</td>
<td>CTTCATTCTGAGG GAGGCAAAGACG TCCAACAC 55</td>
<td>429</td>
<td>Intron 16, Exon 16, Intron 16, partial 3′ UTR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The genotype frequencies for TT/TC in the Saanen, Maltase, Alpine, Damascus, Halep and Kilis populations were 0.85/0.15, 0.83/0.17, 1/0, 0.73/0.27, 0.70/0.30 and 0.76/0.24, respectively. There were no significant differences among populations in the distribution of genotypes. The allele distribution of exon 16 locus of \( DGAT1 \) gene in all populations were agreement with Hardy-Weinberg equilibrium by the Chi-square test (\( P >0.05 \)).

For the determine of relationships between genotype TT and TC and milk traits in goat populations, statistical analyses were used. No significant statistical results were founded in milk yield, fat and lactose values with TT and TC genotypes were detected (\( P >0.05 \) (Table 3).

In spite of the fact that no polymorphisms were detected in Saanen, Maltase, Alpine, Damascus, Halep and Kilis goat breeds, milk traits were analysed. According to milk analyse results, milk yield (\( P <0.001 \)), fat (\( P <0.05 \)), protein (\( P <0.001 \)) and lactose (\( P <0.05 \)) values were found as statistically important. Saanen goat breed has shown highest milk yield (\( P <0.001 \)), Kilis goat breed has highest fat percentage (\( P <0.05 \)), Maltase goat breed has highest protein percentage (\( P <0.001 \)) and Halep goat breed has highest lactose (\( P <0.05 \)) values when compared to other goat breeds (Table 2).

To confirm the exon 16 goat \( DGAT1 \) gene PCR-RFLP results, 20 PCR products representing unique banding patterns were sequenced in both directions in an ABI 310 DNA sequencer and sequences were analysed with Bioedit software and Blast in NCBI (National Centre for Biotechnology Information).

As a sequence results, the comparison between nucleotide sequences of DQ380250 (goat \( DGAT1 \) gene) showed heterozygous point (g.273T>C) was detected in sampled population, except that Alpine goat breed. This variation point g.273T>C was found in intron 16 and can be determined to \( NlaIII \) restriction enzyme (Fig. 2).
the exon 8, exon 16 and exon 17, including intron 6, exon 7, intron 7, exon 8, intron 8 and exon 15, intron 15, exon 16, intron 16 and partially 3‘ untranslated region (UTR) in goat DGAT1 gene and investigate to its possible association genotypes with milk traits in Saanen, Maltase, Alpine, Damascus, Halep and Kilis dairy goat breeds. Another aim of this study was to investigate whether the K232A SNPs in bovine DGAT1 gene exist and affect with milk traits in sampled goat populations.

Angiolillo et al. (2007) has been sequenced a 1552 bp fragment of the goat DGAT1 cDNA, which include coding sequence exon1–17 and a genomic fragment covering exons 12 to 17. As a result of Angiolillo et al. study, polymorphism of the goat DGAT1 gene appear to be rather limited and they did not detect presence of K232A polymorphism in Spanish goats. In compatible with our finding, Angiolillo et al. was determined only T to C substitution at the intron 16 of goat DGAT1 gene and C variant was found to minority allele shown at very low frequencies (0.062–0.109) and could be used as a marker in association studies with milk traits. Miltiadou et al. (2010) were investigating whether the K232A SNPs in bovine DGAT1 exist in Damascus and Machaeras goat breeds. Their results indicated that, K-allelic genotype was fixed in Damascus and Machaeras goat breeds and K232A polymorphism did not detected in any of sampled goat breeds.

Sharma et al. (2011) performed to PCR, SCCP and sequencing methods to examine of polymorphism within intron 15, exon 16, intron 16, partially 3‘ end of exon 15 and 5‘ end of exon 17 DGAT1 gene in Barbari, Beetal and Ganjam goat breeds. Researchers found two substitutions in intron 16 in DGAT1 gene in the Indian goat breeds, which are g.4G>A and g.123T>C. In agreement with our result, g. 123T>C transition was the same in our finding substitution point (g.273T>C) and all sampled goat breeds were found monomorphic for the T nucleotide residue.

In the present study, the SNP of intron 16 of DGAT1 gene was identified and detected for the fourth time in goat breeds after the first report of Angiolillo et al. (2007) in the Murciano-Granadina, Malaguena and Saanen goat breeds and second report of Miltiadou et al. (2010) in the Damascus and Machaeras goat breeds and third report of Sharma et al. (2011) in Barbari, Beetal and Ganjam goat breeds. Here we first describe an NlaIII PCR-RFLP method for detecting T-to-C mutation in intron 16 goat DGAT1 locus: CATG- to -CACG.

In the current study, the K232A variation of cattle, located in exon 8, was not found in Saanen, Maltase, Alpine, Damascus, Halep and Kilis goat breeds, where all individuals were homozygous for the K-alleles encoding

Table 3. Associations of DGAT1 intron 16 genotype with milk traits in sampled goat breeds

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Fat</th>
<th>Protein</th>
<th>Lactose</th>
<th>Milk yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>3.965 A</td>
<td>3.341 A</td>
<td>4.812 A</td>
<td>574.6 A</td>
</tr>
<tr>
<td>TC</td>
<td>3.812 A</td>
<td>3.203 A</td>
<td>4.780 A</td>
<td>571.9 A</td>
</tr>
<tr>
<td>p value</td>
<td>0.480 NS</td>
<td>0.100 NS</td>
<td>0.742 NS</td>
<td>0.822 NS</td>
</tr>
</tbody>
</table>

NS, nonsignificant.

![Fig. 2. DNA Sequence of genotypes TC at the 273 locus and detected variation points in goat DGAT1 gene exon 16 region.](image)

![Fig. 3. Agarose gel electrophoresis band patterns after digestion with Alul endonuclease within the exon 17 sequence of the goat DGAT1 gene; lane M: 100bp DNA marker; lane 1: TC genotype (429bp, 372 bp, and 57 bp); lane 2–4: CC genotype (429 bp) As one small bands (57 bp) were invisible on 4% agarose gel; only two bands were visible for TC genotype.](image)
Lysine. In agreement to our findings, according to information of literature no one has reported the presence of to date K232A mutation in goat breeds. The maintenance of a larger pool of DGAT1 alleles in cattle than in man or goats might be due to the fact that several of these bovine alleles, and not only the K232A polymorphism, are positively selected by their favourable associations with milk traits (Angiolillo et al. 2007, Kühn et al. 2004).

We observed that biallelic polymorphism was found with restriction endonuclease NCOL in intron 16 and this polymorphism was not detected Alpine goat breeds, whereas no polymorphism was found Ald in exon 17 in all sampled goat populations. Genotype frequency analysis showed that no significant difference among six populations of Saanen, Maltase, Alpine, Damascus, Halep and Kilis, indicating that genetic variation is not significant difference among Kilis and Saanen, Maltase, Alpine, Damascus, Halep goat breeds.

Milk fat content has an important effect on cheese yield and firmness as well as on cheese flavour and colour (Angiolillo et al. 2007, Lamberet et al. 2001). Kilis goat breed showed highest fat percentage compared to other goat breeds. SNP in exon 16 DGAT1 gene functional variations needs to be confirmed. The effect of this SNP of DGAT1 on milk traits or milk quality traits in these goat breeds should be further investigated. SNP in exon 16 that might be used as a marker in association studies to determine whether genetic variation at the goat DGAT1 exon 16 locus has any quantitative effect on mammary gland and milk yield. Although not expect to have any functional effect, this T-to-C SNP could be useful as a genetic marker in association studies to detect influence milk fat content and milk traits.

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REFERENCE


