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Polymorphism of goat DGAT*1* 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 *EaeI, NlaIII* and *AIu*l restriction enzymes for exon 8, exon 16 and 17, respectively. No polymorphisms were detected at the *EaeI and AIu*l 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 (exon1–17) 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.

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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, 270, 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' untranslated region (UTR) in *DGAT1* gene. Primer pairs and their annealing temperatures are shown in Table 1. For primer design, Primer3 software (Rozen and Skaletsky 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 $MgCl_2$, 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 2% agarose gels in parallel with a 100 bp DNA marker.

Genotyping: PCR product of 20 µl were digested separately with 10U *EaeI,* 10U *NlaIII* and and 10U *AIu*l for exon 8, exon 16 and 17 respectively at 37°C overnight. PCR products of digestion were resolved by electrophoresis on a 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 (χ^2) 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 *AIu*l 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 lenght (genotype CC) in sampled populations, only a 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 intron 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

Primer pair	Forward primer	Reverse primer	Annealing temparature (°C)	Amplicon size (bp)	Covered region
Exon 8	CCACCATTCTCTG CTTCCCA	GGCGAAGAGGAA GTAGTAGAG	53	514	Partial exon 5, Intron6, Exon 7, Intron 7, Exon 8, Intron 8
Exon 16	CCCAGACACTTCT ACAAGCC	TGCCCGATGATGA GTGACAG	54	401	Exon 15, Intron 15, Exon 16, Intron 16, partial exon 17
Exon 17	CTTCTTCCATGAGG TGAGTGCA	GAGGCAAAGCAG TCCAACAC	55	429	Intron 16, Exon 16, Intron 16, exon 17, partial 3' UTR

Table 2. Means±SD of milk traits in sampled goat populations

Milk traits	Goat breeds						
	Saanen	Halep	Kilis	Maltase	Alpine	Damascus	P value
Fat (%)	3,896^{AB}	3,959^{AB}	4,296^{A}	3,416^{AB}	3,272^{B}	3,778^{AB}	0,030*
Protein (%)	3,192^{B}	3,385 ^{AB}	3,187^{B}	3,691^{A}	3,286^{AB}	3,139^{B}	0,000***
Lactose (%)	4,783^{AB}	4,998 ^{A}	4,666 ^{AB}	4,457 ^{B}	4,967 ^{AB}	4,749 ^{AB}	0,040**
Milk yield (kg)	781,9 ^{A}	426,4 ^{D}	346,4 ^{E}	620,9 ^{C}	688,9 ^{B}	575,3^{C}	0,000***

*,**,*** Means of goat milk traits with different letters are significantly different P<0.05, P<0.01, P<0.001 respectively.

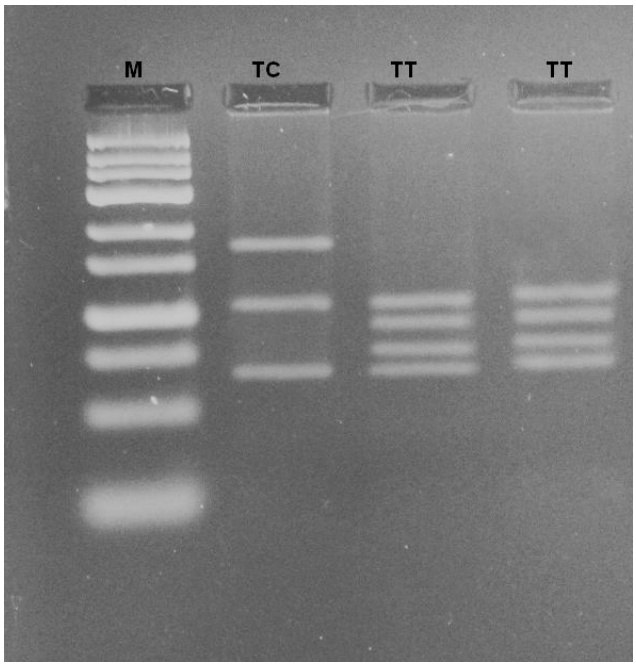

Fig. 1. Agarose gel electrophoresis band patterns after digestion with *NlaIII* endonuclease within the exon 16 and its flanking region sequence of the goat *DGAT1* gene; Lane M: 25bp Low range DNA marker; Lane 2: genotype TC (178 bp, 106bp, 67 bp, 27 bp and 22 bp); Lane 3–4: TT genotype (106 bp, 100 bp, 78 bp, 67 bp, 27 bp and 22 bp). As one small bands (27 and 22 bp) were invisible on % 4 agarose gel only three bands were visible for TC genotype and four bands were visible for genotype TT.

located at intron 16. After digestion with *NlaIII* endonuclease two genotypes were detected: TT and TC. However, genotype TC was not shown Alpine goat breeds. Allele T contained the restriction site for *NlaIII* and resulted in 100 and 78 bp fragments, whereas the absence of the restriction site in the C allele resulted in a single 178 bp fragment. Allele T restriction site resulted in 106 bp, 100 bp, 78 bp, 67 bp, 27 bp and 22 bp fragments, whereas C allele occurred the absence of the restriction site in the 297–300 position resulted in a five fragments 178 bp, 106 bp, 67, 27 bp and 22 bp (Fig.1). Therefore, genotype TT and TC demonstrated six and five bands, respectively. As a result, a biallelic polymorphism was found with restriction endonuclease *NlaIII.* Allel frequencies for Saanen, Maltase, Alpine, Damascus, Halep and Kilis breeds were 0.92, 0.93, 1, 0.86, 0.85 and 0.88 respectively for allele T; 0.08, 0.07, 0, 0.13, 0.15 and 0.12 respectively for allele C. 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).

Many association studies were applied the *DGAT1* gene as a marker for milk yield and milk fat and protein percentages in cattle and allele-K in exon 8 sequence was associated with higher fat yield and both fat and protein percentage, while the allele-A was responsible for higher milk yield. Only a few studies were carried out to find *DGAT1* gene polymorphisms associated with milk production in goat breeds. In the current study we amplified

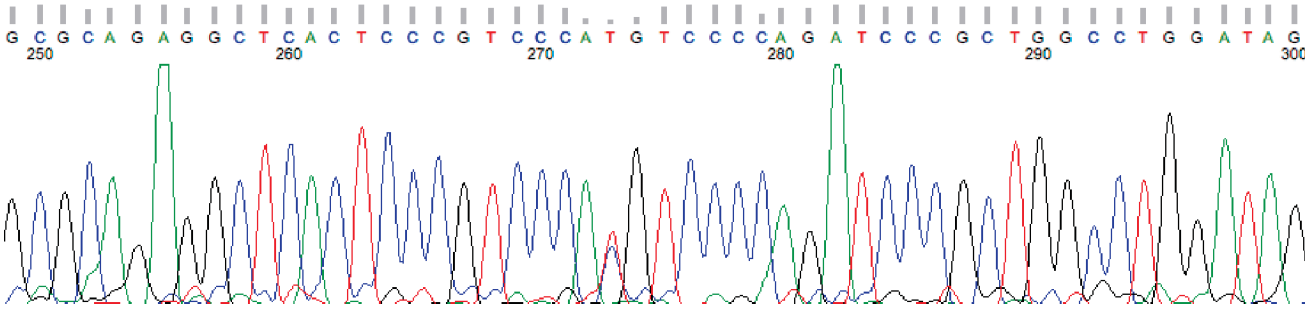

Fig. 2. DNA Sequence of genotypes TC at the 273 locus and detected variation points in goat *DGAT1* gene exon 16 region.

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

Genotypes	Milk traits			
	Fat	Protein	Lactose	Milk yield
TT	3,965 ^{A}	3,341 ^{A}	4,812 ^{A}	574,6 ^{A}
TC	3,812 ^{A}	3,203 ^{A}	4,780 ^{A}	571,9 ^{A}
p value	0,480 ^{NS}	0,100 ^{NS}	0,742 ^{NS}	0,822 ^{NS}

NS, nonsignificant.

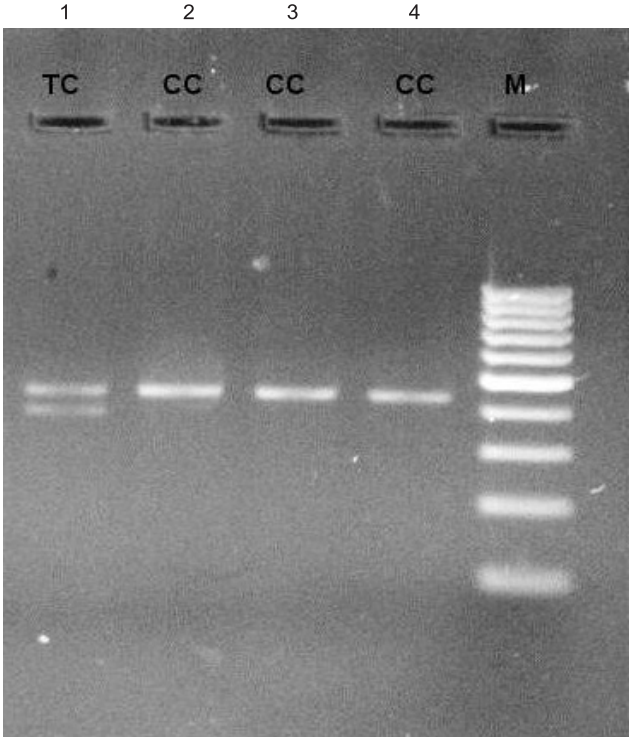

Fig. 3. Agarose gel electrophoresis band patterns after digestion with *Alu*I endonuclease within the exon 17 sequence of the goat *DGAT1* gene; lane M: 100bp DNA marker; lane 1: TC genotype (429bp, 372 bp, and 57 bp); lane 2–4: CC genotype (429 bp) As one small bands (57 bp) were invisible on 4% agarose gel; only two bands were visible for TC genotype.

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, exon 17 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

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 *NlaIII* in intron 16 and this polymorphism was not detected Alpine goat breeds, whereas no polymorphism was found *Alu*I 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

Akcapinar H. 2000. *Sheep Breeding*. Ankara: Ismat Press, Turkey. (in Turkish)

An X P, Song S G, Hou J X, Zhu C M, Peng J X, Liu X Q, Xiao W P, Zhao H P, Bai L, Wang J G, Song X Y and Cao B Y. 2011.Polymorphism identification in goat *DGAT2* gene and association analysis with milk yield and fat percentage. *Small Ruminant Research* **100**: 107–12.

Angiolillo A, Amills M, Urrutia B, Domenech A, Sastre Y, Badaoui B and Jordana J. 2007. Identification of a single nucleotide polymorphism at intron 16 of the caprine acyl-coenzyme A: diacylglycerol acyltransferase 1 (*DGAT1*) gene. *Journal of Dairy Research* **74**: 47–51.

Furbass R, Winter A, Fries R and Kuhn C. 2006. Alleles of the bovine *DGAT1* variable number of tandem repeat associated with a milk fat QTL at chromosome 14 can stimulate gene expression. *Physiological Genomics* **25**: 116–20.

Grisart B, Coppieters Farnir F, Karim L, Ford C, Berzi P, Cambisano N, Mni M, Reid S, Simon P, Spelman R, Georges M and Snell R. 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine *DGAT1* gene with major effect on milk yield and composition. *Genome Research* **12**: 222–31.

Kühn C, Thaller G and Winter A. 2004. Bininda-Emonds OR, Kaupe B, Erhardt G, Bennewitz J, Schwerin M, Fries R. Evidence for multiple alleles at the *DGAT1* locus better explains a quantitative trait locus with major effect on milk fat content in cattle. *Genetics* **167**: 1873–81.

Lamberet G, Delacroix-Buchet A and Degas C. 2001. Intensity of initial lipolysis in goats' milk and the perception of 'goaty' aroma in the cheese. *Proceedings of the Technical Symposium, 7th International Conference on Goats: Recent Advances on Goat Milk Quality Raw Material for Cheesemaking.* 130–39 (Ed. ITPLC). Poitiers, France

Marshall M O and Nudsen J. 1997. Biosynthesis of triacylglycerols containing short-chain fatty acids in lactating cow mammary gland. Activity of diacylglycerol acyltransferase towards shortchain acyl-CoA esters. *Journal of Biochemistry* **81**: 259–66.

Miltiadou D, Tzamaloukas O, Genethliou N, Papachristoforou C, Chatziplis D and Orford M. 2010. Do establish SNPs affecting bovine milk traits exist in other dairy ruminant. *Small Ruminant Research* **88**: 72–74.

Rozen S and Skaletsky H. 2000. Primer3 on the www for General Users and for Biologist Programmers, Bioinformatics Methods and Protocols. *Methods in Molecular Biology*. pp. 365–86. (Eds) Krawetz S, Misener S. Humana Press, Totowa.

Sambrook J and Russell D W. 2011. *Molecular Cloning: A Laboratory Manual.* 3rd edn, pp.1–99. Cold Spring Harbor Laboratory Press, New York.

Sharma R, Pandey A K, Maitra A and Mishra B P. 2011. Established SNPs in *DGAT1* gene of exotic goat is absent in indigenous goat breeds. *Indian Journal of Animal Sciences* **81**: 621–23.

Spelman R J, Ford C A, McElhinney P, Gregory G C and Snell R G. 2002. Characterization of the *DGAT1* gene in the New Zealand dairy population. *Journal of Dairy Science* **85**: 3514–17.

Winter A, Kramer W, Werner F A, Kollers S, Kata S, Durstewitz G, Buitkamp J, Womack J E, Thaller G and Fries R. 2002. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl- CoA: diacylglycerol acyltransferase (*DGAT1*) with variation at a quantitative trait locus for milk fat content. *Proceedings of the National Academy of Sciences* **99**: 9300–05.

Wu X L, MacNeil M D, De S and Xiao Q J. 2005. Evaluation of candidate gene effects for beef backfat via Bayesian model selection. *Genetics* **125**: 103–13.

Yeh F, Yang R C and Boyle T. 2000. Popgene (v.1.32), Microsoft Windows-based freeware for Population Genetic Analysis.

Yen C L E, Stone S J, Koliwad S, Harris C and Farese R V. 2008. *DGAT* enzymes and triacylglycerol biosynthesis. *Journal of Lipid Research* **49**: 2283–301.