

GENETIC ASSOCIATION BETWEEN THE VARIATION OF DGAT1 GENE AND MILK PRODUCTION TRAITS IN KHALKHALI GOATS

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Abstract

The quantity of milk and milk fat and proteins are particularly important traits in dairy livestock. However, little is known about the regions of the genome that influence these traits in goats. This study was performed to investigate genetic variation and association analysis of diacylglycerol acyltransferase 1 (DGAT1) gene on important traits in Khalkhali dairy goats. PCR-SSCP method and Sanger sequencing were used for genotyping. In total, three different SSCP patterns (I, II, III with frequency 0.35, 0.56 and 0.10 respectively) were identified that leads to cause four mutations in exon 17 of DGAT1 gene. The mutations T21153G, C21154G cause changes serine to glycine amino acids and A21172C leads to change in Aspartic acid to Alanine amino acid and one mutation A21194T was silent. Association analysis indicated that haplotypes were related with milk yield and protein percentage of Khalkhali goat ($p < 0.05$), but there is no relationship with milk lactose and solid matter percentage ($p < 0.05$). Our findings indicate that polymorphisms of the caprine DGAT1 gene might be important genetic factors influencing milk traits, and the results of this study provide valuable information about the markers applicable in marker-assisted selection programs in goat breeding and production.

Key words: milk traits, DGAT1, polymorphism, SSCP, Khalkhali goats

1. INTRODUCTION

Milk production traits are the most important economic traits in the dairy industry, including milk yield, fat yield, protein yield, fat percentage and protein percentage (Spelman et al. 1996), that influenced by minor genes (Ashwell et al. 2001). In the past several decades, quantitative trait locus (QTL) mapping, candidate gene analysis and genome-wide association studies have been widely applied to identify the potential causal genes or mutations for milk yield and composition (Georges et al. 1995; Andersson 2009; Schennink et al. 2009). In general identification and validation of the genetic markers of quantitative traits are the initial step to establish a MAS system.

Diacylglycerol acyltransferases (DGATs) include two major gene families shown to encode DGATs, DGAT1 (type-1) and DGAT2 (type-2) (Winter et al., 2003; Ganji et al., 2004). DGAT1 gene was the first identified gene encoding a protein with DGAT activity. A missense mutation (Lys232Ala) in DGAT1 has been shown to be significantly associated with variation in milk fat percentage in cattle (Winter et al., 2002). Generation of viable DGAT1-knockout mice revealed that DGAT-like activity is found in other enzymes encoded by other genes and led to the detection of DGAT2 (Smith et al., 2000; Mossinger et al., 2010). DGAT1 and DGAT2 were demonstrated as two unrelated proteins that exhibited DGAT activity through different pathways, but the DGAT1 gene had the highest expression level in the small intestine, whereas the DGAT2 gene had high expression levels in the liver, white adipose tissue and mammary gland (Cases et al., 2001; Yamazaki et al., 2005). DGAT activity was found in a variety of tissues; relatively higher DGAT activities were found in liver, adipose tissue, small intestine and lactating mammary gland (Cheng et al., 2004).

The wide distribution of DGAT enzyme supports the notion that it participates in lipoprotein assembly, energy storage, dietary fat absorption, and milk production (Cheng et al., 2004). Thaller et al. (2003) reported DGAT1 gene significantly affected intramuscular fat content (IMF) in beef cattle.

In a different country, many reports investigation the characteristic of goat milk, have been focused intensively on milk protein polymorphisms. There is a great variation among the different breeds of goats when it comes to polymorphism distributions. With this in view, many goat breeds have been extensively investigated. However, there exists no information on DGAT1 genetic variation in indigenous Iranian Khalkhali goat. Indigenous goats are important for subsistence economic in rural condition. Therefore the purpose of this study was to identify genetic polymorphism at the partial sequence of DGAT1 gene in indigenous Khalkhali goat breeds.

2. MATERIALS AND METHODS

2.1. Samples and DNA Extraction

A total of 120 unrelated individuals from the Khalkhali goat breed were randomly collected in 4 different flocks in the North West of Iran according to guidelines of the Iranian Council of Animal Care. About 4 mL of blood was collected from the jugular vein using vacutainer tubes containing EDTA. Total genomic DNA was purified from whole blood using Exgen kit and the quality of extracted DNA was checked using 0.8 % Agarose gel electrophoresis.

Milk samples were obtained from Khalkhali goat at the first and second lactation stage. Animals were bred ad libitum access to pasture grass of Iran (37°36'58"N 48°31'31"E) and grazed on natural pasture and milked twice a day (07:00 and 19:00 h). Individual milk samples were collected from morning milking and divided into 2 fractions and were analyzed for fat, protein, lactose and dry matter content..

2.2. PCR Amplification, Sequencing, and Statistical analysis

A 427 bp fragment of exon 17 of the caprine DGAT1 gene was amplified using following primers set: Forward 5'- GCA TGT TCC GCC CTC TGG -3' and Reverse 5'- GGA GTC CAA CAC CCC TGA -3'.

Amplification was carried out in 25 µl volume reaction containing 1.2 mM MgCl₂, 0.2 mM dNTP 1.5U Taq DNA polymerase, 0.15 µM of forward and reverse primer and 100 ng genomic DNA. The thermal profile consisted of denaturation at 95°C for 5 min, followed by 33 cycles of 94°C for 30s, annealing at 59 °C for 30s and extension at 72°C for 60s, with a final extension step at 72°C for 10 min.

Purified PCR products were sequenced under standard condition using sanger sequencing (Sanger et al. 1977). Chromas Ver.2.33, (<https://technelysium.com.au/wp/chromas/>) was used to edit the Sequence data. The obtained sequences were analyzed and compared using MEGA 6.0 (Tamura et al. 2013). The genotype frequency of single nucleotide polymorphisms (SNPs) was determined by direct counting methods. Nucleotide BLAST program at NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used for sequence homology searches in public databases.

Fixed model analysis of milk composition was performed using the GLM procedure of SAS 9.2. The model included Herd, SNP genotype and litter size as a fixed effect abse on following model:

$$Y_{ijkl} = \mu + H_i + L_j + G_k + e_{ijkl}$$

Where, y_{ijkl} is the milk traits measured on $ijkl$ th animal (Fat, protein, lactose, solid material percentage), μ is the mean for the entire population, h_i is the fixed effect associated with i th herd ($i = 1, 2, 3$ and 4), l_j is the fixed effect associated with j th lactation ($j = 1, 2$), g_k is the fixed effect associated with k th genotype ($k = 1$ and 2) and e_{ijkl} is the random error.

Data were presented as least squares means with the associated standard error. The significance of deviations was verified with the Tukey-Kramer test. For inferring intraspecific phylogenies, Median Joining Network analysis^[11] was generated by Network V.4.6.1.1 (<http://fluxus-engineering.com/>).

3. RESULTS AND DISCUSSION

Diacylglycerol acyltransferase (DGAT) is a key enzyme that catalyzes the final and rate-limiting step of triglyceride synthesis (Cases et al., 2001; Wakimoto et al., 2003). Both DGAT1 and DGAT2 genes code proteins with DGAT activity (Lardizabal et al., 2001). Studies have shown that DGAT1 significantly affected milk volume, milk protein and milk fat yield in dairy cattle (Spelman et al., 2002). PCR amplicons with the expected size (approximately 309 bp) were obtained from goat DNA using a set of the DGAT1 primers (Fig. 1). Three unique PCR-SSCP banding patterns were detected for exon 1. These banding patterns represent two different polymorphic sites in exon 17 of DGAT1 gene in khalkhali goat.

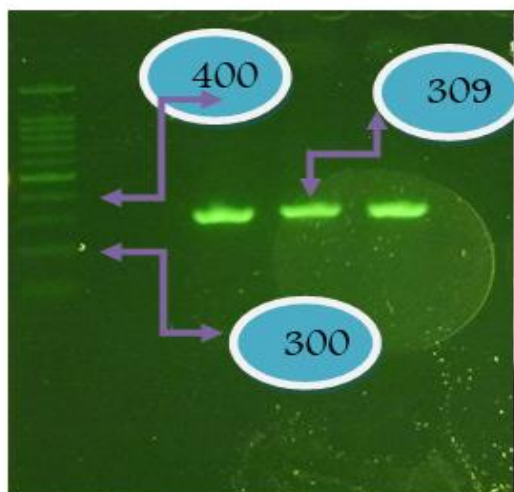


Fig. 1. PCR amplicons with the expected size of DGAT1 gene in Khalkhali goat

The fragment of 309 bp in exon 17 of DGAT1 gene was amplified and sequenced using Sangers sequencing. Alignment analysis of sequences after the quality check showed a polymorphic site of transversion change type that leads to substitute G to T, G to C, C to A and T to A nucleotide in position 110, 111, 129 and 151 respectively (Table 1). Based on the LT221856.1 NCBI reference mutation were placed in 21153, 21154, 21172 and 21194 position respectively. Nonsynonymous and synonymous nucleotide diversity, was observed in the sequences (Table 1).

Table 1. Mutation Observed in Diacylglycerol acyltransferase 1 gene.

Loci	Nucleotide changes	Mutation Type	Code	Amino Acid Changes	Loci base on LT221856.1
110	G to T	Synonymous	GGC to TCC	Serine to Glycine	21153
111	G to C	None-synonymous			21154
129	C to A	None-synonymous	GCT to GAT	Aspartic Acid to Alanine	21172
151	T to A	Synonymous	ACT to ACA	Threonine	21194

DNASP analysis indicated that the selected region (1-309) within sequences from 7 different species have 309 sites, excluding gaps. 18 singleton variable sites were observed in the species. The nucleotide diversity (p) and the average number of nucleotide differences (k) was 0.651 and 11.73 for all sequences were higher than the values in *khalkhali goats* (p=0.08, K=1.33). Distinct differentiation of the species could be concluded based on the high genetic diversity of the DGAT1 gene. The polymorphic information and haplotype diversity of the MYF5 gene for *species* are listed in Table 2.

The haplotype diversity between species was more than 0.66, indicating most abundant genetic diversity between species.

Table 2. Population statistics between Species based on DGAT1 gene.

Row	Species	Sequence No.	Single Loci No.	Haplotype No.	Haplotype Diversity	Average Difference Nucleotide	Nucleotide Diversity
1	Goat	13	18	8	0.91	12.67	0.65
2	African Goat	13	18	7	0.88	12.14	0.67
3	Capra Hircus	13	18	8	0.91	11.73	0.65
4	Capra aegagrus	13	18	10	0.95	12.58	0.70
5	Capra	13	18	8	0.91	12.21	0.68
6	Domestic Goat	13	18	8	0.91	11.79	0.66
7	Khalkhali Goat	10	2	2	0.66	1.33	0.08

A median-joining network constructed of haplotypes for Bactrian and dromedary camels is shown in Figure 1. The phylogenetically central and most frequent be assumed to be the most ancient node in the network as the oldest ancestral allele is the root of the phylogeny, whereas the haplotypes at the tip or periphery of the network regarded as most recently arisen.

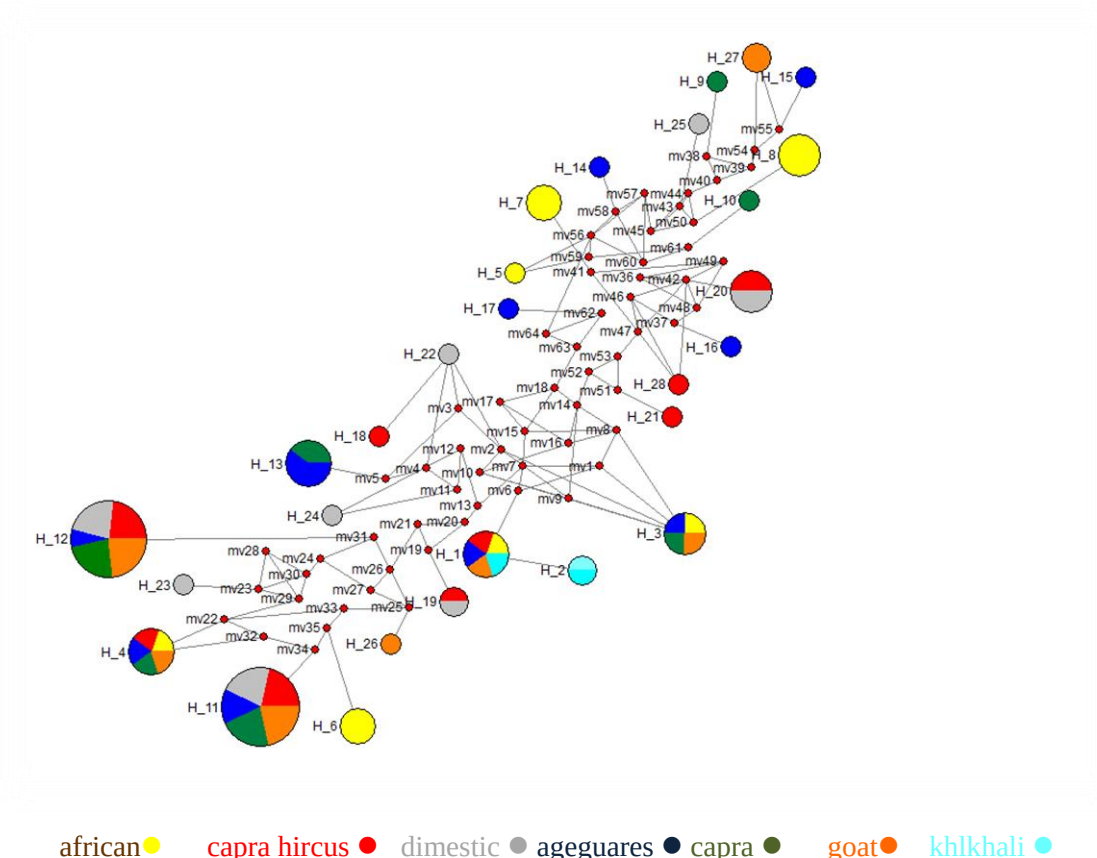


Fig. 2. Network Analysis of Haplotype relation for DGAT 1 gene.

Analysis of DGAT1 gene showed differentiation between goat species. Phylogenetic tree and median-joining network constructed with haplotypes between species was shown in Fig 2 and Fig 3 respectively. *Capra hircus* family was more difference to each other, and distributed in different haplogroups. Based on phylogeny analysis of DGAT1 gene indicated admixture distribution of species of goat and we could not categorize base on this gene. This gene is conserve then it could not be useful for phylogeny analysis.

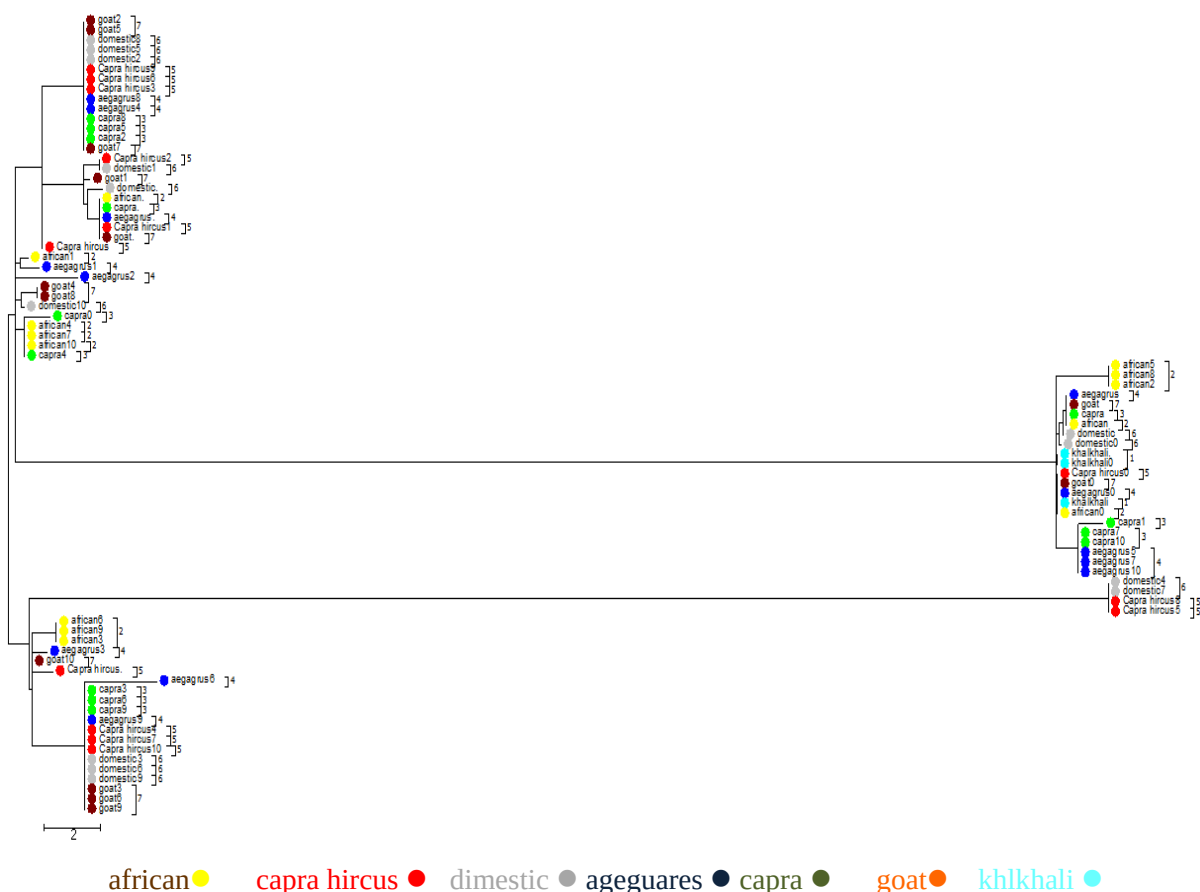


Fig. 3. Phylogenetic tree between species using maximum likelihood.

DGAT1 plays a fundamental role in TAG synthesis by using diacylglycerol and fatty acyl CoA as substrates (Coleman et al. 2000). In cattle, a non-synonymous substitution in DGAT1 influences enzyme activity having a major effect on milk composition (Grisart et al. 2002; Winter et al. 2002). These features make DGAT1 an interesting candidate gene to explain the phenotypic variation of fat percentage and other milk related traits in other ruminant species such as goats.

Table 3. Association between Haplotype Identification and Milk traits in Khalkhali Goat using DGAT1 gene.

Trait	Genotype	Means	Standard error means (SEM)	P value
Fat %	I	2.99	0.331	0.74
	II	3.13		
Milk Solid material %	I	8.73	0.171	0.392
	II	8.93		
Protein %	I	3.22	0.123	0.049
	II	3.49		
Lactose %	I	4.75	0.096	0.591
	II	4.83		
Milk yield	I	74.07	4.345	0.029
	II	78.07		

Statistical analysis showed that herd has a significant effect on all the traits studied (data not shown) ($p < 0.05$). Genotype at the DGAT1 locus significantly influenced on some milk parameters. Association analysis indicated that haplotypes were related with milk yield and protein percentage of Khalkhali goat ($p < 0.05$), but there is no relationship with milk lactose and solid matter percentage ($p < 0.05$). The II genotypes showed the highest protein, and Milk yield ($P < 0.05$) whereas did not effect on lactose percentages, Fat Percentage and Milk Solid material percentage (Table 3).

4. CONCLUSIONS

In the present study, the relationship between the DGAT1 genotype and milk composition traits has been investigated. The data showed clear relationship between genotype and milk yield and protein milk content like other studies in different species and breeds. The result showed that haplotype II in DGAT1 gene had higher protein percentage and milk yield than I haplotype in Iranian Khalkhali goat, but the frequency of haplotype II was less than Haplotype I.

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