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Mva I PCR-RFLP identifies single nucleotide polymorphism of the alpha-lactalbumin gene in some goat breeds reared in Turkey

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Abstract: Alpha-lactalbumin plays a key role in lactose synthesis in the mammary glands of domestic animals. This study evaluated Hair (188), Saanen (82), Kilis (63), and Honamli (183) goat breeds, with the primary goal of investigating DNA polymorphism of *Mva* I RFLP at exon 3 of the alpha-lactalbumin gene. Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) was employed to genotype a total of 516 goats for *Mva* I polymorphism of alpha-lactalbumin. In the breeds that were studied, digestion of the amplification product with the *Mva* I restriction enzyme revealed 2 alleles (A1 and A2) and 3 genotypes (A1A1, A1A2, and A2A2). Allelic frequencies for Hair, Honamli, Saanen, and Kilis breeds were found to be 0.87, 0.92, 0.87, and 0.85, respectively, for the A1 allele and 0.13, 0.08, 0.13, and 0.15, respectively, for the A2 allele. None of the studied breeds exhibited significant deviation from Hardy–Weinberg equilibrium. Consequently, this study shed light on the polymorphism of alpha-lactalbumin in 4 goat breeds. Another finding of this study was the presence of genetic polymorphism at the alpha-lactalbumin gene in Kilis and Honamli goat breeds, a fact that was not known previously.

Key words: Alpha-lactalbumin, goat, LALBA, PCR-RFLP, polymorphism

1. Introduction

Alpha-lactalbumin (α-LA, LALBA) is a major whey protein found in ruminant milk (1). This calcium metalloprotein influences lactose synthesis by modifying the substrate specificity of UDP-galactosyltransferase in the mammary glands of domestic animals (2,3,4). For this reason, it has potential as a quantitative trait locus for dairy cattle (5). Gordon et al. (6) and Brew et al. (7) were the first to identify the primary amino acid sequence of bovine α-LA. Goat α-LA is a milk whey protein homologous to lysozyme (1,8). With 123 amino acid residues and a molecular weight of 14,200 Da (9,10), it has been used as a model protein in protein folding studies (11,12). The gene that codes lactalbumin has been localized in the fifth chromosome of the goat genome (13) and the transcription unit consists of 4 exons varying in length from 75 nucleotides to 329 nucleotides (8). The structure of the LALBA gene has been studied in rats (14), humans (15), cattle (16), goats (8), and guinea pigs (17), leading to the claim that the gene's organization is similar to that of chicken egg whites (18) as well as human (19) genes that code lysozyme.

Polymorphism of the LALBA gene in ruminants has been most frequently studied in cattle and 3 alleles were initially reported at the protein level for cattle species at this locus (20,21), but polymorphisms were subsequently identified in intron regions as well. The first study to investigate the 4 exons of the LALBA gene and the neighboring regions reported a T→C transition in nucleotide 13 of intron 1, a C→T transition in nucleotide 5 of exon 3, and a C→G transversion in nucleotide 187 of exon 4 (22). The same study reported that the mutation in nucleotide 5 of exon 3 did not cause any change in the amino acid sequence and also that, when compared with the reference sequence in GenBank, the *Mva* I enzyme caused the cutting site to disappear. In a study on Mongolian Cashmere goats and Chinese domestic goat breeds, Lan et al. (23) reported a new C→T transition in nucleotide 7 of exon 3. They also determined that this C→T transition in nucleotide 7 of exon 3 causes L→P amino acid changes and that this is a cutting site for the *Msp* I enzyme. In a study investigating the relationship between cashmere productivity and this mutation in exon 3 of Mongolian Cashmere goats,

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Lan et al. (24) reported that individuals with a heterozygotic TC genotype had greater cashmere productivity than individuals with the other 2 genotypes. Another study carried out on goat breeds raised in China revealed a C→T transition in nucleotide 875 of exon 1, showing that this was related to body size (4). In a study on the LALBA gene in domestic Indian breeds of goats, Jain et al. (3) reported that exons 1, 2, and 3 of the 4 exons were monomorphic while 2 T→C transitions were identified in noncoding nucleotide regions 190 and 263 of exon 4, and that this is a *Mae* III cutting site for region 190.

Various regions in Turkey raise a number of goat breeds, including Angora, Hair, Kilis, Norduz, Honamli, and Saanen. The most prevalent goat breed in Turkey is the Hair goat, which is raised all over the Anatolian peninsula, except along the Black Sea coast (25). The Hair goat is found in a broad area in Turkey that includes the Mediterranean region, the Aegean region, and Southeast Anatolia. The Hair goat usually has a black coat and horns, which is why it is also called the "black goat" in Turkey (25). Its long, coarse hair is not wavy. It is a multipurpose goat breed with regard to meat, milk, and hair (26). The Kilis goat is raised in the provinces of Kilis, Gaziantep, Adıyaman, and Hatay in the southern Anatolian region of Turkey (25). Most male and female goats have horns, but sometimes they have no horns at all. The Kilis goat provides the highest milk yield of any goat in Turkey. The breed's body is strong and its hair is straight, long, and coarse (25). The Kilis goat breed was developed by cross-breeding Syrian goats from Damascus with Hair goats, which are native to Turkey (26,27). The Honamli goat breed is raised in the provinces of Burdur, Konya, and Antalya. Females have elegant horns that curve backwards, while males have horns that are curved at various angles (25). It is raised in the "Teke Region", which includes Antalya, Burdur, and the Taurus Mountains (28). Both male and female goats have horns and the breed features a large, ram-shaped nose. Honamli goats are bred for meat, milk, and hair. Saanen goats are a Swiss breed that is known around the world for superb milk yield (26).

Most studies performed on genetic regions that may be related to economically important characteristics have focused on cattle and sheep. The number of studies carried out on this subject for goat breeds is very limited, both in Turkey and around the world, in spite of how important goats are. The goal of this study was to use the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method to investigate differences in the *Mva* I cutting site in exon 3 of the LALBA gene in samples taken from Hair, Honamli, Kilis, and Saanen goat breeds from Turkey and to describe the variations in alleles and the genotype.

2. Material and methods

2.1. Animal material

A total of 516 blood samples were collected from 4 different goat breeds living in natural habitats. The goats were not blood-related according to breeders' information. To reliably represent genetic variation, examples were collected from different regions. The study evaluated 188 Hair goats (Antalya, Burdur), 82 Saanen goats (Burdur, Ankara, Isparta), 63 Kilis goats (Kilis, Niğde), and 183 Honamli goats (Antalya, Burdur). Approximately 10 mL of blood per goat was collected aseptically from the jugular vein and kept in a tube containing EDTA for DNA isolation.

2.2. DNA isolation

In order to isolate genomic DNA, a genomic DNA isolation kit (GeneJET Genomic DNA Purification Kit) was used according to the manufacturer's protocol.

2.3. PCR and genotyping

The sequences of the forward and reverse primers for amplifying the LALBA gene (accession number DQ673921.1) were as follows: forward 5'-TCATCTAAAAGGCAACAGGTA -3' and reverse 5'-ATAGTGCTGGGGCGAAA -3'. PCR was carried out in 25 µL containing 1.5 mM $MgCl_2$, 200 µM of each dNTP, 5 pmol of each primer, 1X PCR buffer, 1 U of Taq polymerase, and 100–150 ng of goat genomic DNA. PCR conditions were as follows: denaturation at 95 °C for 5 min followed by 33 cycles of denaturation at 94 °C for 30 s, annealing at 55.2 °C for 30 s, and extension at 72 °C for 40 s, with a final extension at 72 °C for 10 min, on an Amplitronyx 6 thermal cycler. The PCR products were digested with FastDigest *Mva* I restriction endonuclease (Thermo Scientific, #FD0554) at 37 °C for 5 min. PCR products and fragments were separated by electrophoresis on 2% and 4% agarose gels, respectively.

2.4. Data sets and statistical analysis

Direct counting was used to estimate genotype and allele frequencies of genetic variants of the LALBA gene *Mva* I. A chi-square statistic was used to check whether the populations were in Hardy–Weinberg equilibrium. PopGene32 software was used to carry out all statistical analyses (www.ualberta.ca/~fyeh/Pop32.exe).

3. Results

The 268-bp product of exon 3 of the LALBA gene was digested with restriction endonuclease *Mva* I in 4 goat breeds. Two alleles (A1 and A2) were identified at the *Mva* I site together with 3 genotypes (A1A1, A1A2, and A2A2). By using PCR, a 268-bp fragment was successfully amplified (Figure). RFLP polymorphism within the goat

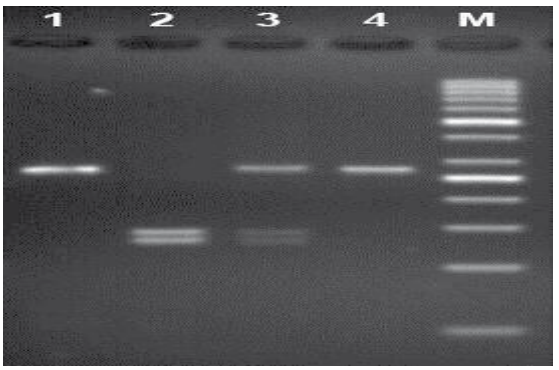

Figure. Electrophoresis of PCR amplifications of LALBA gene (268 bp, Lane 1) and RFLP of goat LALBA gene after digestion by *Mva* I of animals with A1A1 (Lane 2; 140 bp/128 bp), A1A2 (Lane 3; 268 bp/140 bp/128 bp), and A2A2 (Lane 4; 268 bp) genotypes. Lane M, molecular marker (50-bp DNA ladder).

LALBA gene detected by *Mva* I restriction enzyme is illustrated in the Figure, showing the fragments obtained for the LALBA/*Mva* I polymorphism: 140 and 128 bp for the A1A1 genotype; 268, 140, and 128 for A1A2; and 268 for A2A2. The Table shows the allelic and genotypic frequencies of LALBA gene polymorphism for Hair, Honamli, Kilis, and Saanen goats.

Allelic frequencies for Hair, Honamli, Saanen, and Kilis breeds were found to be 0.87, 0.92, 0.87, and 0.85, respectively, for the A1 allele and 0.13, 0.08, 0.13, and 0.15, respectively, for the A2 allele. On the other hand, frequencies were 0.76, 0.84, 0.78, and 0.73 for A1A1; 0.23, 0.15, 0.18, and 0.24 for A1A2; and 0.01, 0.01, 0.04, and 0.03 for A2A2, respectively. No significant deviation from Hardy–Weinberg equilibrium was observed in the breeds that were studied.

4. Discussion

This study evaluated the genetic polymorphism of the α-LA gene in Hair, Honamli, Kilis, and Saanen goat breeds. Two alleles (A1 and A2) were identified at the *Mva* I site together with 3 genotypes (A1A1, A1A2, and A2A2). For some goat breeds, A1 and A2 were found to exist at the LALBA locus (3,22,23,29). All the breeds that were studied revealed similar results. Similarly, 3 genotypes were observed in Hair, Honamli, Saanen, and Kilis goats at the LALBA locus. For all the samples that were studied, the most frequently occurring genotype was A1A1.

Öner et al. (29) studied LALBA polymorphism in goats with 27 Hair goats, 18 Gökçeada goats, and 13 Saanen goats. The researchers found that the A1A2 genotype was less frequent than A1A1, and they did not find the A2A2 genotype. In contrast, the present study did identify the A2A2 genotype, albeit at a lower frequency than A1A1 and A1A2. Furthermore, Öner et al. (29) showed that the A2 allele was less frequent at the LALBA locus than the A1 allele. Similarly, the frequency of the A2 allele was 0.11, 0.20, and 0.25 for Girgentana, Red Syrian, and the local population reared in the province of Naples, respectively, with genotype distributions in Hardy–Weinberg equilibrium (22). This was consistent with the result of the present study in all breeds. Lan et al. (23) studied Inner Mongolia White Cashmere, Guanzhong dairy, Guizhou Black, Matou, and Banjiao goat populations and found that the frequencies of the A2 allele in those populations were 0.017, 0.024, 0.024, 0.023, and 0.020, respectively. In contrast, the same study found a frequency of 0.000 for Xinong Saanen dairy, Laoshan dairy, Leizhou, and Guizhou White goats raised in China, with genotype distributions in Hardy–Weinberg equilibrium. Genetic polymorphisms in lactalbumin have been identified in domesticated animals both at the protein (11,12) and DNA (3,4) levels. LALBA polymorphism has been found

Table. Allele and genotype frequencies of the LALBA gene for the *Mva* I site in Hair, Honamli, Saanen, and Kilis goat breeds.

Breed	n	Genotype						Allele Frequency		χ^2 (df = 1)	P-value
		A1A1		A1A2		A2A2		A1	A2		
		Obs. (Exp.)	F	Obs. (Exp.)	F	Obs. (Exp.)	F				
Hair	188	142 (143.01)	0.76	44 (41.98)	0.23	2 (3.01)	0.01	0.87	0.13	0.441	0.51^{NS}
Honamlı	183	154 (154.19)	0.84	28 (27.62)	0.15	1 (1.19)	0.01	0.92	0.08	0.036	0.85^{NS}
Saanen	82	64 (62.29)	0.78	15 (18.42)	0.18	3 (1.29)	0.04	0.87	0.13	2.957	0.09^{NS}
Kilis	63	46 (45.37)	0.73	15 (16.26)	0.24	2 (1.37)	0.03	0.85	0.15	0.399	0.53^{NS}
Total	516	406 (404.69)	0.79	102 (104.61)	0.20	8 (6.69)	0.01	0.89	0.11	0.323	0.57^{NS}

F: frequency; NS: nonsignificant.

at the DNA level in different Turkish goat breeds (29). However, no variation has been reported across the board for LALBA polymorphisms at the DNA level, especially for Honamli and Kilis goats.

The present study found that the Hair, Honamli, Saanen, and Kilis goat breeds in Turkey have genetic polymorphism in the LALBA gene. In particular, this study has also showed the existence of genetic polymorphism of the LALBA gene in Honamli and Kilis goats. Alpha-lactalbumin plays a key role in lactose synthesis in the mammary glands of domestic animals. Thus, even if association studies of these single nucleotide polymorphisms with the milk protein traits have not been done in this breed, it is very important to conserve each variant in a gene pool. The associations between genetic variations of milk composition and its production are of great importance to the dairy industry. Further determination is required to identify the link with economic traits such as milk yield.

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