

## Genetic polymorphism of the *CSN1S1* gene in the Greek-indigenous Skopelos goat

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### Abstract

Goat milk has been an invaluable asset for Mediterranean countries. Milk yield and composition is greatly affected by polymorphisms in the *CSN1S1* gene which encodes for the  $a_{s1}$  casein fraction of milk. Currently, about 18 alleles have been described which are associated with four levels of  $a_{s1}$  casein. In the present work, we investigated the genetic structure of *CSN1S1* alleles in a population of Greek-autochthonous Skopelos goats. The high-protein yield A\* (A, G, I, H) and B\* (B1, B2, B3, B4 and C) alleles were detected at higher frequencies (0.56 and 0.59, respectively) compared to the low-protein alleles (F, 0.06 and E, 0.07). The presence of the null N allele was detected at a frequency of 0.26. Genotyping of the Skopelos goat population did not show carriers of the O1 allele. The assessment of the *CSN1S1* genetic variability could be useful for the genetic improvement of the Skopelos goat, a breed that is well-adapted to low-input farming conditions.

### Introduction

In Greece as well as in many countries around the Mediterranean, goat milk is primarily used for cheese production. Therefore, the economic revenue of goat milk production depends greatly not only on milk yield but also on protein and fat content (Park and Haenlein, 2007). Extensive polymorphisms of the caprine gene that encodes the  $a_{s1}$  casein (CN) fraction (*CSN1S1*) have been reported to be associated with milk yield and milk composition in many goat breeds. At least 18 different alleles have been identified so far (Devold et al., 2010) that are classified in 4 groups: strong alleles (A, B1, B2, B3, B4, B', C, H, L, M) are associated with increased levels of  $a_{s1}$ -CN in milk (3.6 of g/L), intermediate alleles (E, I) with 1.6 g/L, weak alleles (F, G) with 0.6 g/L, and null alleles (O1, O2, N) which are characterized by absence of  $a_{s1}$ -CN in milk.

The Skopelos breed is an important autochthonous dairy goat that is reared mainly in the Sporades islands and in the mainland of central Greece. It represents a highly homogenous population comprised of about 11,000 animals. The breed is well adapted to dry, low-input systems, which are characterized by sparse vegetation and poor scrublands. The purpose of this study was to evaluate the genetic variability of the  $a_{s1}$ -casein locus in the Skopelos breed.

### Material and methods

Blood samples were collected from 238 Skopelos breed goats. The animals were grouped in 2 herds that were located in the Sporades islands of Greece. Both herds were reared using traditional low-input farming. DNA was extracted from 200 $\mu$ L of blood using the GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific Inc.).

Genotyping at the *CSN1S1* locus was performed using allele-specific PCR and PCR-RFLP protocols. For identifying the *CSN1S1* A\* (including A, G, I, H), B\* (including B1, B2, B3, B4 and C), F and N alleles the PCR-RFLP method described by Ramunno et al. (2000, 2005) was followed. The identification of E and O1 alleles was performed using allele-specific PCR (AS\_PCR) according to Torres-Vázquez et al. (2008) and Cosenza et al. (2003), respectively.

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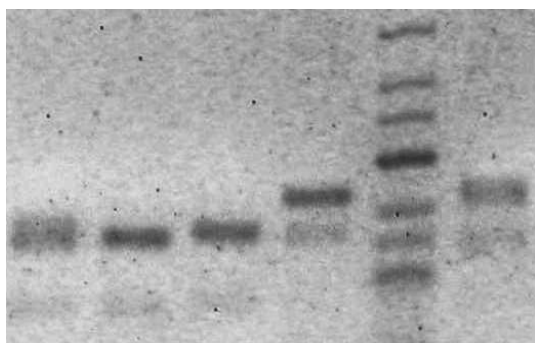
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## Results

Polymorphisms at the *CSN1S1* locus have been extensively characterized in several economically important breeds as well as in indigenous breeds from different countries. However, to date, casein variability at the genomic level in the indigenous Greek goat breeds has not been assessed.

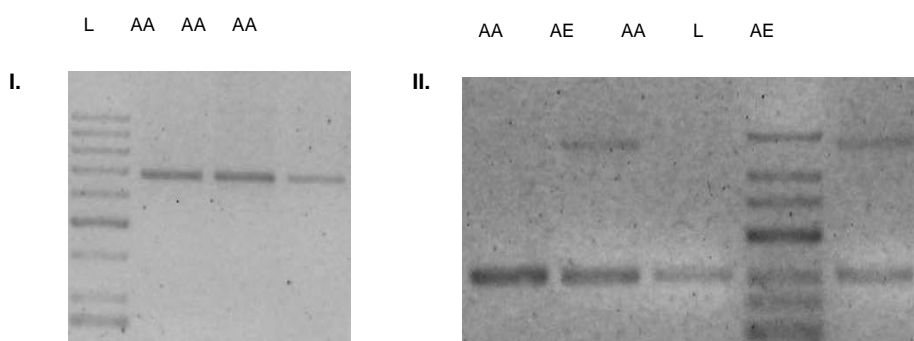
The region of the caprine *CSN1S1* gene between nucleotides 202 and 420 that includes part of the eighth intron, the ninth exon and part of the ninth intron, was amplified and subsequently digested with the restriction endonuclease *XmnI*. Observed genotypes are presented in Figure 1.

A\*B\* A\*A\* B\*B\* B\*N L A\*F



**Figure 1. PCR-RFLP analysis of A\*, B\*, F, and N alleles of *CSN1S1* in representative Skopelos goat individuals. Amplified fragments were digested with *XmnI* for 5 hours and then electrophoresed in 2.5% agarose-TAE gel. The molecular size marker (L) used was GeneRuler Low Range DNA Ladder (Thermo Scientific Inc.).**

The most common genotype was A\*B\* (0.24) followed by B\*B\* (0.22) and A\*A\* (0.21). These genotypes are associated with production of milk with high protein content and more favourable cheese-making properties in many European goat breeds. The most frequent alleles observed in the Skopelos goat population were A\* (0.56) and B\* (0.59). *CSN1S1* E and F alleles were identified at a very low frequency (0.07 and 0.06, respectively). However, the N null allele was detected at a frequency of 0.26, a frequency similar to the Neapolitan goat and goats of French origin. The O1 null allele was not identified in any of the tested animals (Figure 2).



**Figure 2. I. AS-PCR analysis of *CSN1S1* 01 allele. 01 allele was not identified in the Skopelos population tested. A depicts all non-01 alleles. II. AS-PCR analysis of *CSN1S1* E allele. A depicts all non-E alleles. Amplified fragments of the *CSN1S1* gene were electrophoresed in 2% agarose-TAE gel. The molecular size marker (L) used was GeneRuler 50 bp DNA Ladder (Thermo Scientific Inc.).**

## Discussion

The high frequency of the strong alleles A\* and B\* in Skopelos goats indicate that this breed possesses an allelic combination associated with high milk protein yield. Studies on the effect of *CSN1S1* polymorphism on milk yield and composition are currently under way by our group. The *CSN1S1* genotype information will be

valuable to use in selection strategies in order to breed animals for higher milk yield and protein content. On the other hand, information on the null (N) allele will be useful in creating animal niches that produce milk for specialized applications (e.g. milk with low allergenic potential). Analysing the molecular diversity of local Greek breeds that are reared using traditional low-input farming systems will establish a new horizon for the conservation and sustainability of the indigenous goat population.

### **Suggestions to tackle with the future challenges of organic animal husbandry**

Breeds of dairy goats, well-adapted to harsh environments, play a key role to sustainable milk production under low-input and organic farming systems in less favoured areas. Under these systems, genetic selection based on milk quality traits may favour the more efficient utilization of restricted resources in order to produce high-quality products.

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