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Genetic polymorphism at the *CSN1S1* gene in Girgentana dairy goat breed

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Abstract. The aim of this work was to evaluate the variability of the αs_1-casein locus in the endangered Girgentana dairy goat breed in order to define genetic improvement and a conservation program for this breed. The study was performed on 200 dairy goats by means of different PCR protocols. The most frequent alleles were A (0.590) and F (0.290) followed by B (0.065) and N (0.047). *CSN1S1* E allele was identified with a very low frequency (0.008). The most common genotype was AF (0.365) followed by AA (0.340). The high frequency of the strong genotypes is associated with the production of milk with high fat and protein content and with optimal technological properties. In Girgentana goat breed, the *CSN1S1* genotype information could be utilised in selection strategies for milk protein content and milk yield, in order to select genetic lines for the production of 'drinking milk' using weak and null genotypes, and for niche products using strong genotypes.

Additional keywords: Girgentana goat, milk production.

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Introduction

In the milk of ruminants, more than 95% of proteins are synthesised by six structural genes, four caseins (αs_1, β, αs_2 and κ-caseins) and two whey proteins (α-lactalbumin and β-lactoglobulin). The four caseins represent ~80% of milk proteins. Among Ca-sensitive caseins (αs_1, β, and αs_2), the αs_1 fraction is the most extensively investigated in goat (Martin *et al*. 2002; Rijnkels 2002). At genomic level, it is encoded by a single autosomal gene (*CSN1S1*) mapped on caprine chromosome 6 and clustered with genes of the other casein fractions (*CSN1S2*, *CSN2* and *CSN3*) on a DNA fragment of ~250 kb (Grosclaude *et al*. 1987; Ferretti *et al*. 1990; Threadgill and Womack 1990; Leroux and Martin 1996). The *CSN1S1* gene has a 17.5-kb-long transcriptional unit composed by 19 exons, which vary in length from 24 to 382/388 bp, and 18 introns from 90 to 1685 bp (Martin *et al*. 1999; Ramunno *et al*. 2004). So far, at least 17 codominant alleles have been identified, which are associated with different expression levels of αs_1-casein in milk. A first group of alleles (A, B1, B2, B3, B4, C, H, L, M) are associated with a high content of αs_1-casein (~3.5 g/L), alleles I and E are associated with an intermediate content (~1.1 g/L), and alleles D, F, and G with a low level (~0.45 g/L) of this protein in milk. Alleles *CSN1S1* N, 01 and 02 are 'null' alleles and have been associated with the absence of αs_1-casein in milk (Grosclaude *et al*. 1987, 1994; Chianese *et al*. 1997; Martin *et al*. 1999; Bevilacqua *et al*. 2002; Ramunno *et al*. 2005).

In goat, the B1 allele is the original one from which the A-type (A, G, I, H, 01, and 02) and B-type alleles (B2, B3, B4, C, E, F, L and D) originated (Chianese *et al*. 1997). The M allele is considered a result of an interallelic recombination event between the A- and B-type alleles (Bevilacqua *et al*. 2002). A similar event was also proposed for the origin of N allele (Ramunno *et al*. 2005).

Most of the mutational events responsible for the formation of such alleles have been identified. The A, B1, B2, B3, B4, C, G, H, L and M alleles originated from single nucleotide substitutions responsible for amino acid substitutions (Chianese *et al*. 1997; Martin *et al*. 1999; Bevilacqua *et al*. 2002). While the molecular event characterising the I allele is unknown (Chianese *et al*. 1997), the E allele is characterised by the insertion of a DNA segment (Long Interspersed Nuclear Element, 457 nucleotides long) between the 124th and the 125th nucleotide of the 19th exon (Jansà Pérez *et al*. 1994). The D allele is characterised by an internal deletion of 11 amino acid residues with respect to A allele (Leroux *et al*. 1992). The F allele is characterised by the deletion of the 23rd nucleotide of the 9th exon and by the presence of short insertion of 11 and 3 bp inside the 9th intron (Leroux *et al*. 1992; Ramunno *et al*. 2000, 2005). Moreover, the N allele, analogously to the F allele, is characterised by the same exonic mutation, but without the insertion of 11 and 3 bp in the subsequent intron (Ramunno *et al*. 2002). The null allele 01, the true null allele, is characterised by the deletion of a DNA segment of ~8.5 kb, starting from the 181 nucleotide of the 12th intron, and including the last 7 exons of the gene (Cosenza *et al*. 2003), while a large insertion, so far uncharacterised, is the mutational event responsible for the 02 allele (Martin *et al*. 1999).

The goat *CSN1S1* gene represents an excellent model for demonstrating that the major part of the variability observed in the αs_1-casein content in the goat milk is due to the presence of autosomal alleles at a single structural locus (Ramunno *et al*. 2005). The extensive polymorphism at αs_1-casein locus has been

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shown to affect not only the quantity of casein in goat milk, but also the structural and nutritional characteristics and technological properties of milk. In fact, polymorphism associated with a quantitative variability in casein synthesis has a significant effect on coagulation properties, micelle size and mineralisation, cheese yield, and sensory attributes (Ramunno *et al*. 2007). Another important aspect to be considered is the growing importance of goat milk in the infant diet, due to the reports that goat's milk in some cases is less allergenic than cow's milk. The major protein in cow milk is αs_1-casein, which is not produced by human beings (Haenlein 2004). Goat milk proteins have many significant differences in their amino acid compositions from the milk of other mammalian species, especially in relative proportions of the various milk proteins and in their genetic polymorphisms (Jenness 1980; Boulanger *et al*. 1984; Addeo *et al*. 1988; Ambrosoli *et al*. 1988). Goat milk may differ genetically by having either less ('Null' type) or more ('High' type) content of this protein. Null types have shorter rennet coagulation time, less resistance to heat treatment, curd firmness is weaker, pH is higher, protein and mineral contents in milk are lower, and cheese yields are less than in high types (Ambrosoli *et al*. 1988). This indicates and may explain significant differences to cow milk in digestion by infants and patients (Mack 1953).

The Girgentana goat is an ancient Sicilian goat breed reared in Southern Italy for its good dairy production. Average milk production was 224 ± 66 L in the first lactation, and 320 ± 109 L for later lactations (AIA 2011). According to morphology, this breed probably came from Afghanistan and the Himalaya regions (Portolano 1987). Due to sanitary policies the size of the Girgentana population decreased almost 90% in 20 years. In 1983, the population consisted of 30 000 Girgentana goats, now only 651 goats are reared (ASSONAPA 2012). Over recent years this breed has become almost extinct, in part as a consequence of the marked decrease in fresh goat milk consumption. Therefore, it could be interesting to evaluate the possibility of revitalising interest in the milk produced by this breed in order to regain an important economic role in the production of quality 'drinking-milk' requested for particular food products, such as milk for infants, and in the production of niche products.

The aim of this work was to evaluate the genetic polymorphisms of the αs_1-casein gene in the endangered Girgentana dairy goat breed in order to define genetic improvement and conservation program for this breed, considering that preservation of breeds in danger of extinction could be achieved by establishing economic reasons for their survival.

Materials and methods

A total of 200 individuals, all females, from the Girgentana goat breed were randomly collected in 15 different flocks located in different areas of Sicily. Samples were collected from 10 to 15 unrelated individuals per herd. About 10 mL of blood was collected from the jugular vein using vacutainer tubes containing EDTA as anticoagulant. Genomic DNA was extracted from buffy coats of nucleated cells using a salting out method (Miller *et al*. 1988). The concentration of extracted DNA was checked using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The *CSN1S1* A*/01, B*/E, F and N alleles were simultaneously investigated by PCR-RFLP using *Xmn*I (Ramunno *et al*. 2000). This protocol allowed the identification of F and N alleles, but did not distinguish allele A* from 01, and allele B* from E. Allele-specific-PCR was used for the detection of the *CSN1S1* E (Dettori *et al*. 2009) and *CSN1S1* 01 alleles (Cosenza *et al*. 2001, 2003). PCR and PCR-RFLP products were analysed directly by electrophoresis on agarose gel stained with ethidium bromide.

The exact *P*-value associated with the null hypothesis of Hardy–Weinberg equilibrium was estimated using GENEPOP version 4.0.11 (Rousset 2008). The program performed a probability test using a Markov Chain method (1000 dememorisation steps, 100 batches, and 1000 iterations per batch). Moreover, GENEPOP was used to calculate genotype and allele frequencies and fixation index F_{is} (Weir and Cockerham 1984).

Observed (H_o) and expected (H_e) heterozygosity (Nei 1978) under Hardy–Weinberg equilibrium were calculated using the GENETIX software package version 4.05 (Belkhir *et al*. 1996–2004).

Results and discussion

Table 1 shows the genotype and allele frequencies at *CSN1S1* locus in Girgentana breed. The A* indicated A, G, I, and H alleles while B* indicated B1, B2, B3, B4, and C alleles. The most frequent alleles were A* (0.590) and F (0.290) followed by B* (0.065) and N (0.047). Allele E was identified in three animals and in heterozygous condition, therefore with a very low frequency (0.008). The *CSN1S1* 01 allele was not found in the analysed Girgentana goat individuals. These results were in agreement with those reported by Gigli *et al*. (2008), except that these authors did not report the presence of allele E in this breed. Our results showed for the first time the presence of *CSN1S1* E allele within the Girgentana goat breed. Allele E was absent in other goat breeds reared in Southern Italy and showed low frequency in Maltese (Gigli *et al*. 2008) and in Sarda goat breeds (Dettori *et al*. 2009). Furthermore, while the strong alleles appeared more frequently in the autochthonous goat population reared in Southern Italy, allele E was more frequent in Spanish (Jordana *et al*. 1996), French (Ramunno *et al*. 1994)

Table 1. Genotype and allele frequencies at *CSN1S1* locus in Girgentana goat breed

Genotypes	*n*	Frequency	Allele	Frequency
AA	68	0.340	A	0.590
AB	13	0.065	B	0.065
BB	4	0.020	E	0.008
AE	2	0.010	F	0.290
AF	73	0.365	N	0.047
BF	3	0.015	–	–
EF	1	0.005	–	–
FF	18	0.090	–	–
AN	12	0.060	–	–
BN	2	0.010	–	–
FN	3	0.015	–	–
NN	1	0.005	–	–

and American (Maga *et al*. 2009) goat breeds. For the A and F alleles, Marletta *et al*. (2005) reported frequency values similar to our results (A = 0.600 vs 0.590, F = 0.040 vs 0.047). We identified *CSN1S1* N with a low frequency (0.047) according to Gigli *et al*. (2008) (0.040). The F and N alleles were characterised by one common molecular event (the deletion of the 23rd nucleotide of the 9th exon) but are associated with different levels of αs_1-casein expression in the milk. The cytosine deletion in N allele is resulting in one-nucleotide frame shift and determines a premature stop codon. The amount of mRNA transcribed by the *CSN1S1* N allele is apparently one-third of that transcribed by the *CSN1S1* F allele and, similar to this one, alternatively spliced transcripts are produced. It has been suggested that a mutation, occurring at –1319 nt of the promoter region, creates an extra putative activator protein (AP-1) binding motif in the sequence of the F allele, which can be responsible for the different expression between alleles F and N (Ramunno *et al*. 2005).

Twelve genotypic classes were found in the Girgentana goat breed. The most common genotype was AF (0.365) followed by AA (0.340) and FF (0.090).

The high frequency of the strong genotypes can be associated with the production of milk with high fat and protein content and with optimal technological properties. In fact, clear and significant differences were observed in the cheese yield with +7.4% between AA and EE milks, and +14.8% between AA and FF milks (Grosclaude and Martin 1997). Moreover, casein concentration is greater when strong alleles are present (Martin *et al*. 2002). Maga *et al*. (2009) in a study conducted on the αs_1-casein content in American dairy goats, using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and two-dimensional gels, showed that milk from FF and EE animals had 35 and 25% less caseins, respectively, than animals homozygous for the strong alleles. The presence of A or B allele in heterozygote condition with either the F or E allele reduced the deficit in casein by only 5–7% compared with AA homozygote condition.

A possible inconvenience of strong and intermediate genotypes has been found in relation with cheese flavour. Cheeses made with milk from these genotypes have less typical goat flavour than those from weak genotypes due to different fatty acid profiles (Delacroix-Buchet *et al*. 1996). Moreover, polymorphism at *CSN1S1* locus influenced milk fatty acid composition, the FF genotype has been associated with low percentages of fatty acids entirely (C12 : 0, C14 : 0, C6-C14) or partially (C18 : 0, odd and branched-chain fatty acid) *de novo* synthesised in the mammary tissues, and with high milk Δ^9 desaturated fatty acids (*cis*-9 C14 : 1, *cis*-9 C16 : 1, *cis*-9 C17 : 1 and *cis*-9 C18 : 1) (Valenti *et al.* 2010).

Table 2 shows the H_o and the H_e heterozygosity, the F_{is} and the Hardy–Weinberg equilibrium probability test values. Girgentana goat breed was in Hardy–Weinberg equilibrium at this locus ($P > 0.05$) (Table 2). The H_o and H_e values (0.545 and 0.563, respectively), and the positive value of F_{is} (0.0058) for Girgentana goat breed showed low genetic diversity compared with results obtained for Alpine ($H_e = 0.787$), Saanen ($H_e = 0.670$) and other goat breeds from Mexico (Torres-Vázquez *et al*. 2008), and higher value compared with Indian goats (Kumar *et al*. 2007).

Table 2. Expected (H_e) and observed (H_o) heterozygosity, fixation index F_{is}, Hardy–Weinberg equilibrium probability test (*P*-value) and standard error (s.e.) values at *CSN1S1* locus in Girgentana goat breed

H_o	H_e	F_{is}	*P*-value ± s.e.
0.545	0.563	0.0058	0.121 ± 0.008

Conclusions

In dairy goat populations, the *CSN1S1* genotypes should be considered in order to incorporate this information in the selection processes, and therefore propitiate an increase on the rate of genetic gain for casein contents and milk yield through gene-assisted selection, compared with classical selection schemes where candidates are sorted according to polygenic estimated breeding value only, without considering known genotypic information for some identified genes (Moioli *et al*. 1998; Dekkers 2004; Sanchez *et al*. 2005). In the Girgentana goat breed, the *CSN1S1* genotype information could be utilised in selection strategies for milk protein content and milk yield, in order to select genetic lines for the production of 'drinking milk' and for niche products.

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