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 \(\alpha_s\)-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 (\(\alpha_s\), \(\beta\), \(\alpha_2\) and \(\kappa\)-caseins) and two whey proteins (\(\alpha\)-lactalbumin and \(\beta\)-lactoglobulin). The four caseins represent \(\approx80\%\) of milk proteins. Among Ca-sensitive caseins (\(\alpha_s\), \(\beta\), and \(\alpha_2\)-caseins), the \(\alpha_s\) fraction is the most extensively investigated in goat (Martin \textit{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 (CSN2, CSN2 and CSN3) on a DNA fragment of \(\approx250\) kb (Grosclaude \textit{et al.} 1987; Ferretti \textit{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 \textit{et al.} 1999; Ramunno \textit{et al.} 2004). So far, at least 17 codominant alleles have been identified, which are associated with different expression levels of \(\alpha_s\)-casein in milk. A first group of alleles (A, B1, B2, B3, B4, C, H, L, M) are associated with a high content of \(\alpha_s\)-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 \(\alpha_s\)-casein in milk (Grosclaude \textit{et al.} 1987, 1994; Chianese \textit{et al.} 1997; Martin \textit{et al.} 1999; Bevilacqua \textit{et al.} 2002; Ramunno \textit{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 \textit{et al.} 1997). The M allele is considered a result of an interallelic recombination event between the A- and B-type alleles (Bevilacqua \textit{et al.} 2002). A similar event was also proposed for the origin of N allele (Ramunno \textit{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 \textit{et al.} 1997; Martin \textit{et al.} 1999; Bevilacqua \textit{et al.} 2002). While the molecular event characterising the I allele is unknown (Chianese \textit{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 (Jansá Pérez \textit{et al.} 1994). The D allele is characterised by an internal deletion of 11 amino acid residues with respect to A allele (Leroux \textit{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 \textit{et al.} 1992; Ramunno \textit{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 \textit{et al.} 2002). The null allele 01, the true null allele, is characterised by the deletion of a DNA segment of \(\approx8.5\) kb, starting from the 181 nucleotide of the 12th intron, and including the last 7 exons of the gene (Cosenza \textit{et al.} 2003), while a large insertion, so far uncharacterised, is the mutational event responsible for the 02 allele (Martin \textit{et al.} 1999).

The goat CSN1S1 gene represents an excellent model for demonstrating that the major part of the variability observed in the \(\alpha_s\)-casein content in the goat milk is due to the presence of autosomal alleles at a single structural locus (Ramunno \textit{et al.} 2005). The extensive polymorphism at \(\alpha_s\)-casein locus has been
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 αs1-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 αs1-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 XmnI (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 Fis (Weir and Cockerham 1984).

Observed (H0) and expected (H0) heterozygosity (Nei 1978) under Hardy–Weinberg equilibrium were calculated using the GENETIX software package version 4.0.5 (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>
<thead>
<tr>
<th>Genotypes</th>
<th>Frequency</th>
<th>Allele</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>0.340</td>
<td>A</td>
<td>0.590</td>
</tr>
<tr>
<td>AB</td>
<td>0.065</td>
<td>B</td>
<td>0.065</td>
</tr>
<tr>
<td>BB</td>
<td>0.020</td>
<td>E</td>
<td>0.008</td>
</tr>
<tr>
<td>AE</td>
<td>0.010</td>
<td>F</td>
<td>0.290</td>
</tr>
<tr>
<td>AF</td>
<td>0.365</td>
<td>N</td>
<td>0.047</td>
</tr>
<tr>
<td>BF</td>
<td>0.015</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EF</td>
<td>0.005</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>FF</td>
<td>0.090</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>AN</td>
<td>0.060</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BN</td>
<td>0.010</td>
<td>–</td>
<td>–</td>
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<tr>
<td>FN</td>
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</tr>
<tr>
<td>NN</td>
<td>0.005</td>
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Polymorphism of the CSN1S1 gene in Girgentana goats

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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 αs1-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 αs1-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 de

sulfate methyltransferase (DMT) activity in the mammary tissues, and with high milk α9 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 H0 and the H0 heterozygosity, the Fis, 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 H0 and H0 values (0.545 and 0.563, respectively), and the positive value of Fis (0.0058) for Girgentana goat breed showed low genetic diversity compared with results obtained for Alpine (H0 = 0.787), Saanen (H0 = 0.670) and other goat breeds from Mexico (Torres-Vázquez et al. 2008), and higher value compared with Indian goats (Kumar et al. 2007).

Conclusions

In dairy goat populations, the CSN1S1 genotypes should be considered in order to incorporate this information in the selection processes, and therefore propagate 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 (Moïoli 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.

Acknowledgements

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References


Table 2. Expected (H0) and observed (Hobs) heterozygosity, fixation index Fis, Hardy–Weinberg equilibrium probability test (P-value) and standard error (s.e.) values at CSN1S1 locus in Girgentana goat breed

<table>
<thead>
<tr>
<th>H0</th>
<th>Hobs</th>
<th>Fis</th>
<th>P-value ± s.e.</th>
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<tbody>
<tr>
<td>0.545</td>
<td>0.563</td>
<td>0.0058</td>
<td>0.121 ± 0.008</td>
</tr>
</tbody>
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