Available online at www.sciencedirect.com
ScienceDirect
Small Ruminant Research 74 (2008) 84–90
Small Ruminant Research
www.elsevier.com/locate/smallrumres

Genotyping at the *CSN1S1 locus* by PCR-RFLP and AS-PCR in a Neapolitan goat population

G. Cosenza, A. Pauciullo, D. Gallo, L. Colimoro, A. D'Avino, A. Mancusi, L. Ramunno *

Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università degli Studi di Napoli "Federico II", Napoli, Italy

Received 26 January 2007; received in revised form 27 March 2007; accepted 28 March 2007
Available online 9 May 2007

Abstract

The goat *CSN1S1* gene has for many years been an excellent model for demonstrating that most of the variability observed in the αs1-casein content in goat's milk is due to the presence of autosomal alleles at a single structural *locus*. Until now, about 17 alleles associated to at least four levels of αs1-casein expression in milk have been described at the *CSN1S1 locus* in the domestic goat (*Capra hircus*). The great importance of goat αs1-casein polymorphism is due to its qualitative as well as quantitative implications.

In the present work five PCR protocols (PCR-RFLPs, AS-PCR) were set up for rapid genotyping of B1, B2*, B3, B4 and C *CSN1S1* alleles, until now detectable only by milk electrophoresis. Application of these protocols, together with previously described methods to identify *CSN1S1* 01, E, M, F, N and A* (*CSN1S1* A, G, I, H) alleles, allow us to define, at DNA level, the genetic structure of the autochthonous goat reared in the province of Naples for the highest number of possible alleles at this *locus*. Monitoring of CSN1S1 variability in the Neapolitan goat population indicates a high frequency of low (F, 0.368) and null (N, 0.227) alleles.

Keywords: Goat; *CSN1S1*; Alleles; PCR-RFLP; AS-PCR

1. Introduction

Among Ca-sensitive caseins (αs1, β and αs2), the αs1 fraction is without doubt the most extensively investigated in goat. Such protein is codified by a single autosomal gene (*CSN1S1*) mapped on chromosome 6 and clustered with genes of the other casein fractions (*CSN1S2*, *CSN2* and *CSN3* for αs2, β and k, respectively) in a DNA stretch of about 250 kb (Leroux and Martin, 1996; Rijnkels, 2002).

So far, at least 16 alleles have been identified, which are associated with different levels of αs1-casein expression in the milk. A first group of alleles (A, B1, B2, B3, B4, C, H, L, M) are related to a normal content of αs1-casein (about 3.5 g/l), whereas alleles I and E are associated to an intermediate content (about 1.1 g/l), and alleles F and G are related to a low level of αs1-casein in the milk (about 0.45 g/l). Alleles *CSN1S1* N, 01 and 02 are 'null' alleles and have been associated with the apparent lack of αs1-casein in milk (Chianese et al., 1997; Martin et al., 1999; Bevilacqua et al., 2002; Ramunno et al., 2005).

Most of the mutational events responsible for the formation of such alleles have already been identified. The B1 allele is the original one from which two

* Corresponding author. Tel.: +39 0812539004; fax: +39 0817762886.
E-mail address: ramunno@unina.it (L. Ramunno).

0921-4488/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.smallrumres.2007.03.010

divergent lineages of alleles originate: A-type (A, 01, 02, I, G, H) and B-type (B2, B3, B4, C, E, F, L) alleles (Chianese et al., 1997; Grosclaude and Martin, 1997). It has been hypothesized that the *CSN1S1* N and M alleles could be originated by an interallelic recombination event between A- and B-type alleles (Bevilacqua et al., 2002; Ramunno et al., 2005). In particular, the alleles associated to a normal content of this casein fraction originated from single nucleotide substitutions responsible for aminoacid substitutions (Chianese et al., 1997; Martin et al., 1999; Bevilacqua et al., 2002). While the molecular event characterizing the I allele is unknown (Chianese et al., 1997), the E allele is characterized by insertion of a DNA segment (LINE, Long Interspersed Nuclear Element, 457 nucleotides long) which took place within the 19th exon (Jansà Pérez et al., 1994). For the G allele the mutational event is a transition (G → A) occurring in the 5′-splice site consensus sequence responsible of an alternatively spliced mRNA characterized by the out-splicing of exon 4 (Brignon et al., 1990; Martin and Leroux, 1994).

The F allele is, instead, characterized by a deletion of the 23rd nucleotide of the 9th exon (Leroux et al., 1992). Cytosine deletion results in a one-nucleotide frameshift and determines a premature stop codon in exon 12 (Leroux et al., 1992; Ramunno et al., 2005). By means of Northern blot analysis the amount of αs1 casein mRNA transcribed from the F allele was estimated to be at least six times lower than the transcribed one from the A allele (Leroux et al., 1992). Furthermore, the F allele was shown to yield multiple alternatively spliced transcripts, of which the most representative mRNA population is characterized by the alternative skipping of exons 9–11 and is responsible as a consequence, for the synthesis of a form of αs1 casein deprived of 37 aa (Leroux et al., 1992; Ramunno et al., 2005). Sequence data and typing results show that the *CSN1S1* N allele is characterized, like the *CSN1S1* F allele, by the same exonic mutation. 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 (Ramunno et al., 2005). 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 of alleles F and N (Ramunno et al., 2005).

The 01 allele, the true null allele, is characterized by the deletion of a DNA segment of about 8.5 kb starting from the 181st nucleotide of the intron 12, and including the last 7 exons of the gene (Cosenza et al., 2003), while a large insertion, so far uncharacterized, is the mutational event responsible for the 02 allele (Martin et al., 1999).

Furthermore, recent analysis of the *CSN1S1* gene promoter showed a transition G → A in position −1623. This mutation, besides characterizing the N allele, would also characterize the 01 allele, being polymorphic for the A allele. The latter result would suggest the existence of a second A allele (called A2) from which the two null alleles would originate (Ramunno et al., 2005), supporting the idea that alleles 01 and 02 derive from two different A subtypes (Grosclaude and Martin, 1997).

While the *CSN1S1* A and B* (B* = B1, B2, B3, B4, C) alleles appear more frequently in the autochthonous goat population of southern Italy, alleles E and F are more frequent in French, Swiss and Spanish breeds (Ramunno et al., 1994; Jordana et al., 1996; Enne et al., 1997; Grosclaude and Martin, 1997; Caroli et al., 2006).

The aim of the present study is to establish the genetic structure at *CSN1S1 locus* of the Neapolitan goat and to set up methods for the identification of *CSN1S1* B1, B2 B3, B4, C alleles by using PCR-RFLP (PCR-restriction fragment polymorphism) and AS-PCR (allele specific-PCR).

2. Materials and methods

2.1. DNA samples

The research was carried out on 285 individual DNA samples of local Neapolitan goats reared on different farms in the Sorrento Peninsula (southern Italy). Such population is the result of the crossbreed of goat Neapolitan mainly with Saanen, Malta and Alpine goats. The main attitude of the Neapolitan goat is the kids and milk production (approximately 450 l for lactation) (Ciotola and Peretti, 2004). Genomic DNA was extracted from blood leukocytes according to the method of Goossens and Kan (1981).

2.2. Genotyping at goat CSN1S1 locus by PCR based methods

Alleles *CSN1S1* M, E and F, N and 01 were detected by means of ACRS-PCR (amplified created restriction site-PCR) (Bevilacqua et al., 2002), PCR (Jansà Pérez et al., 1994), *Xmn*I PCR-RFLP (Ramunno et al., 2000) and AS-PCR (Cosenza et al., 2003), respectively. Furthermore, based on the nucleotide sequence data, the PCR-RFLP procedure developed for genotyping of *CSN1S1* F and N alleles using *Xmn*I allows the *CSN1S1* A* allele (*CSN1S1* A, G, I, H) to be discriminated (Ramunno et al., 2000).

In order to identify the goat carriers of *CSN1S1* C, B3, B2* (*CSN1S1* B2, L) and B1 alleles, genotyping methods based on PCR-RFLP were developed, whereas AS-PCR was set up

Table 1
Oligonucleotide primers, positions and restriction enzymes for PCR-RFLPs and AS-PCR assays

CSN1S1 alleles	Position nt[b]	Primers sequence (5′–3′)	Genotyping
C	5048–5070 Complementary to: 5222–5241	Forward: AACAGCACTGTTAAATGTATAAT Reverse: TCATCAGTTAAGCTACACAA	*Hph*I[a]
B4	16914–16931 Complementary to: 17287–17304	Forward: AGAACAGTGGAAAGACTG; AGAACAGTGGAAAGACTA Reverse: CCCACACTGCATTCTAAT	AS-PCR
B3	12064–12084 Complementary to: 12274–12294	Forward: TTAGTTTCCCATTCTTTACTC Reverse: GAAGCTCTAACATGATTTGAT	*Dde*I[a]
B2	5995–6016 Complementary to: 6284–6304	Forward: TTCAAATGGAAAAACATTCTCC Reverse: GTCAAATGTATAGGTACAGAT	*Mnl*I[a]
B1	10463–10483 Complementary to: 10752–10773	Forward: GAAAAGAGAACATGTACTTTG Reverse: CATCTTCCTTTTGAATGTACTT	*Mnl*I[a]

[a] Restriction enzymes used for PCR-RFLP genotyping.
[b] Numbering of primers agrees with the nucleotide sequence of the goat *CSN1S1* gene (EMBL Acc. No. AJ504710).

Table 2
Thermal amplification programs for (a) PCR and (b) AS-PCR

Cycle	Denaturation	Annealing	Extension
(a) PCR			
1	97 °C–2 min	55 °C–45 s	72 °C–45 s
31	94 °C–45 s	55 °C–45 s	72 °C–45 s
1	94 °C–45 s	55 °C–45 s	72 °C–10 min
(b) AS-PCR			
1	97 °C–2 min	55.8 °C–45 s	72 °C–1.5 min
29	94 °C–45 s	55.8 °C–45 s	72 °C–1.5 min + 4 s each
1	94 °C–45 s	55.8 °C–45 s	72 °C–10 min

to detect the carriers of *CSN1S1* B4 allele. Primer sequences and the thermal amplification conditions are reported in Tables 1 and 2, respectively. PCR was carried out by using Gene Amp PCR System 2400 (Perkin-Elmer). The *CSN1S1* A, G, I, H and *CSN1S1* B2 and L alleles were gathered in *CSN1S1* A* and *CSN1S1* B2* groups, respectively, since they are indistinguishable with the methods developed in this study.

The 25 μl reaction mix for each PCR product comprised: 100 ng of genomic DNA, 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% Triton X-100, 3 mM $MgCl_2$, 5 pmol of each primer, dNTPs each at 400 μM, 2.5 U of *Taq* DNA Polymerase (Promega, Madison, WI), and 0.04% BSA. Digestion of 17 μl of each PCR amplification was accomplished with 10 U of the specific endonuclease for 5 h at 37 °C following the supplier's directions for buffer conditions. PCR and digestion products were analysed directly by electrophoresis in 1.5% TBE agarose gel (Bio-Rad) in IX TBE buffer and stained with ethidium bromide.

3. Results and discussion

The G → C transversion at the 22nd nucleotide of exon 10 which differentiates *CSN1S1* B1 allele from A-type (A, 01, 02, I, G, H) alleles (Table 3) is responsible for the creation of the *Mnl*I restriction site (↓NNNNNNNGAGG). Consequently, the amplified fragments relative to the *CSN1S1*B1 and *CSN1S1* "non-B1" alleles are characterized by the presence and absence, respectively, of the restriction site. Therefore, by means of *Mnl*I digestion of PCR products, including the 10th exon and part of the 11th exon of the goat *CSN1S1* gene, *CSN1S1* "non-B1" homozygous individuals show a single undigested fragment of 311 bp, whereas *CSN1S1* B1 homozygous individuals have two fragments of 125 and 186 bp (Fig. 1).

By contrast, a restriction site of the same endonuclease (CCTCNNNNNNNN↓) is altered by the T → C transversion occurring at the eighth nucleotide of exon 4 from which the *CSN1S1* B2 allele originated (Table 3). Therefore, digestion with this endonuclease of the amplified DNA fragment (310 bp), contained this exon and flanking region, shows a single undigested fragment for the *CSN1S1* B2 allele and two fragments of 77 and 233 bp for "non-B2" alleles (Fig. 1).

The *CSN1S1* B3 allele derives from the B2 allele as a consequence of a G → A transition occurring at the 14th nucleotide of exon 12 (Table 3). This mutation removes a *Dde*I endonuclease restriction site (C↓TNAG). *Dde*I digestion of a PCR product of 231 bp spanning the 12th exon and flanking regions, would allow carriers for the presence of adenine to be identified. As a consequence, the PCR product, uncut in the presence of guanine, is now restricted to two fragments of 97 and 134 bp (Fig. 1).

However, the mutation which discriminates allele B4 from B3 (a nucleotide substitution A → G at the 139th nt of exon 17) (Table 3) does not alter or create any restriction site. Hence, a method based on allele-specific PCR (AS-PCR) was developed in order to distinguish the carriers of the *CSN1S1* B4 allele. Using specific

Table 3
Variants of the goat *CSN1S1* gene

CSN1S1 alleles	Nucleotide and amino acid position							
	16th/17th nt (3rd exon)	8th nt (4th exon)	23th nt (9th exon)	22th nt (10th exon)	14th nt (12th exon)	139th nt (17th exon)	From 181th nt (12th intron)	Between 124th and 125th nt (19th exon)
B1	CA His^{8}	T Leu^{16}	C Ser^{66}	G Glu^{77}	G Arg^{100}	A Thr^{196}		
B2*		C Pro^{16}						
B3		C Pro^{16}			A Lys^{100}			
B4		C Pro^{16}			A Lys^{100}	G Ala^{196}		
C	AT Ile^{8}	C Pro^{16}			A Lys^{100}	G Ala^{196}		
A*				C Gln^{77}				
F		C Pro^{16}	Deleted					
N			Deleted	C Gln^{77}				
M		C Pro^{16}	T Leu^{66}	C Gln^{77}				
E		C Pro^{16}			A Lys^{100}	G Ala^{196}		Insertion LINE
01				C Gln^{77}		Deleted	Deletion ∼8.5 kb	Deleted

The *CSN1S1* B1 allele is the original one from which the different alleles originate. Nucleotides present at polymorphic positions and corresponding amino acid changes in each variant are indicated. Nucleotides and amino acid positions in the protein are also indicated. *CSN1S1* A* = A, G, I, H, 02; *CSN1S1* B2* = B2, L.

primers for AS-PCR, the obtained amplicon length is 391 bp and it includes part of exon 17 and the next intron. The two allele-specific forward primers differ in the last nucleotide at 3′-end (A → G). Thus, for the *CSN1S1* ≡4 homozygote samples, PCR amplification is successful only using forward primer with guanine at 3′-end, whereas homozygote samples for "non-B4" alleles are successfully amplified only by forward primer with adenine at 3′-end. The heterozygote samples are effectively amplified with both forward primers (Fig. 2).

Finally, the mutation characterizing the *CSN1S1* C allele (double nucleotide substitution, CA → AT, occurring between the 16th and 17th nt of exon 3) (Table 3) alters the restriction site of *Hph*1 endonuclease (↓NNNNNNNTCACC). As shown in Fig. 2, the amplified fragments relative to the *CSN1S1* C and *CSN1S1* "non-C" alleles are characterized by the absence and presence, respectively, of the restriction site. Therefore, by means of PCR-RFLP, *CSN1S1* "non-C" homozygous individuals show two fragments of 83 and 111 bp, whereas *CSN1S1* C homozygous individuals show a single undigested fragment of 194 bp.

The typing of B-type alleles (*CSN1S1* B1, B2*, B3, B4, C) was carried out going backwards from alleles B1

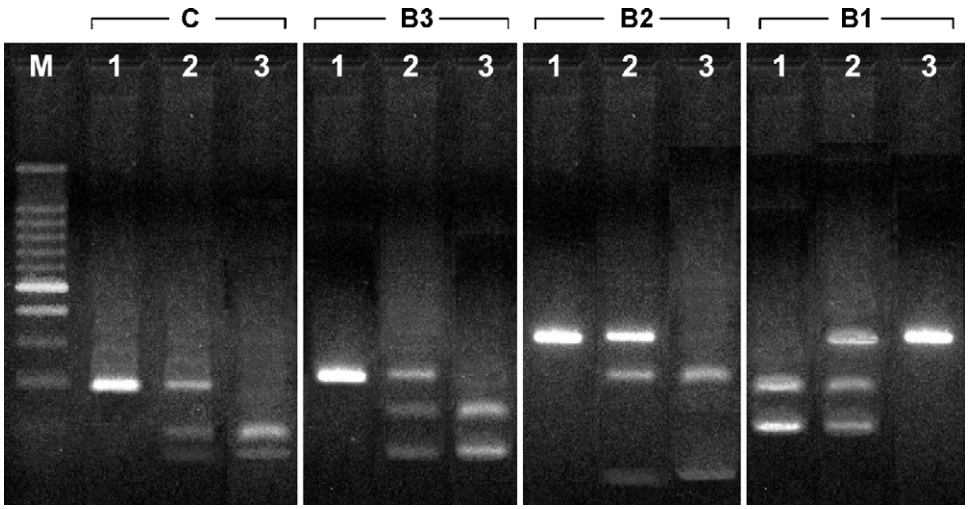

Fig. 1. Typing of *CSN1S1* B1, B2*, B3, C alleles by PCR-RFLP performed by going backwards along the phylogenetic tree. Lines 1 and 3 are homozygous samples for *CSN1S1* C, B3, B2*, B1 and *CSN1S1* "non-C", "non-B3", "non-B2*", "non-B1", alleles, respectively. Lines 2 are heterozygous samples, M is a 100 bp ladder (Promega). *CSN1S1* B2* = B2, L.

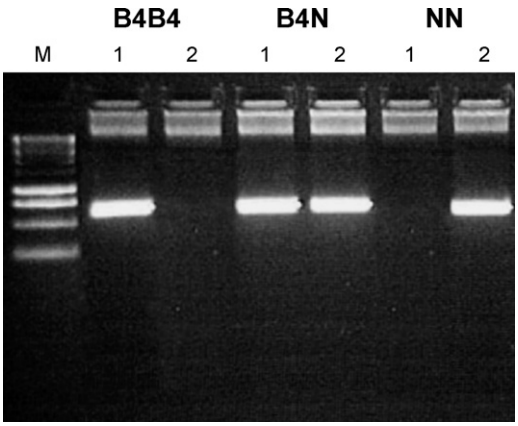

Fig. 2. Typing of *CSN1S1* B4 allele by AS-PCR. Lines 1 are amplicons obtained with a specific forward primer for *CSN1S1* B4 allele, lines 2 are products obtained with a specific forward primer for not *CSN1S1* B4 allele, M is a 100 bp molecular ladder (Promega). *CSN1S1* N = "non-B4" allele.

to C along the phylogenetic tree (Fig. 3). This is was necessary since these alleles originated one by one in successive mutations.

The results of B-type allele genotyping (*CSN1S1* B1, B2*, B3, B4, C) by means of the set up protocols confirmed the origin of each allele as reported in the phylogenetic tree (Fig. 3).

Genotyping the whole population did not show carriers of *CSN1S1* M and 01 allele, and allowed the observation of 26 out of 45 possible genotypes with 9 alleles present in the investigated population. The distribution of the observed genotypes and the frequency of alleles at *CSN1S1 locus* are reported in Table 4. In particular, among the strong alleles of the B-type group (total frequency, 0.18), our research evidenced a higher frequency of B3 (0.072) and B4 (0.085) alleles. In comparison to the other local populations reared in the rest of southern Italy, local Neapolitan goats are characterized

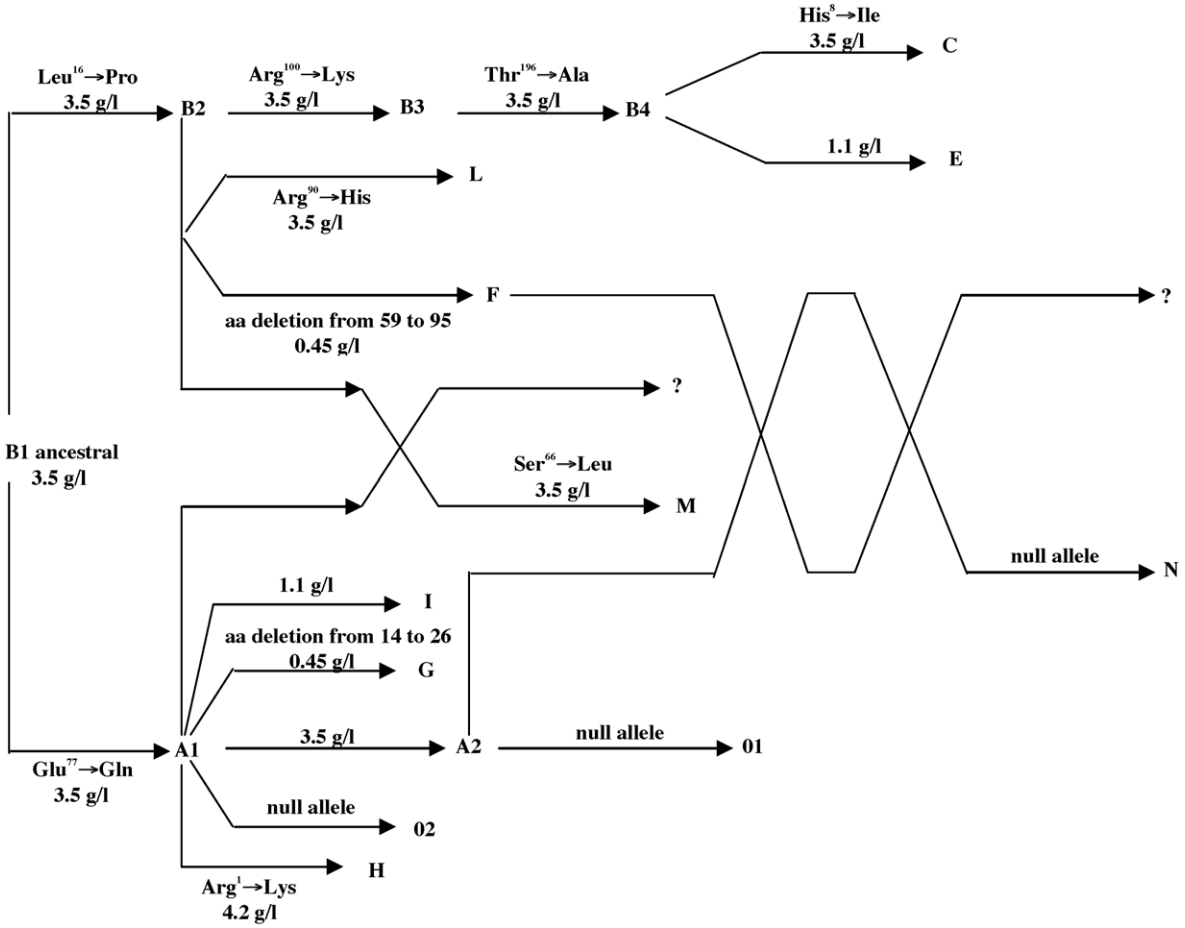

Fig. 3. Phylogeny tree for the goat *CSN1S1* alleles and differences between the corresponding variants. The new proposed phylogeny with respect to those proposed by Chianese et al. (1997) and Bevilacqua et al. (2002), has been enriched with two novel variants (N and A2) reported in Ramunno et al. (2005). The αs1-casein content in milk and the aminoacid differences between variants are also indicated for each variant. The nucleotide mutations are indicated in the text except for I and 02 which are as yet uncharacterized.

Table 4
Number of individuals typed (no.), observed genotypes and allele frequencies at *CSN1S1 locus* in the local Neapolitan goat

Observed genotypes	No.
A*A*	6
A*B3	11
A*B4	2
A*C	2
A*E	2
A*F	34
A*N	18
B1F	4
B2*E	2
B2*F	2
B2*N	2
B3B3	2
B3B4	2
B3E	2
B3F	18
B3N	4
B4B4	11
B4E	2
B4F	18
B4N	4
EE	4
EF	16
EN	15
FF	34
FN	50
NN	18
Total	285

CSN1S1 alleles	Frequency	Genotyping
B1	0.007	*Mnl*I PCR-RFLP[a]
B2*	0.012	*Mnl*I PCR-RFLP[a]
B3	0.072	*Ded*I PCR-RFLP[a]
B4	0.085	AS-PCR[a]
C	0.004	*Hph*I PCR-RFLP[a]
A*	0.142	*Xmn*I PCR-RFLP[b]
M	–	ACRS[c]
E	0.083	PCR[d]
F	0.368	*Xmn*I PCR-RFLP[b]
N	0.227	*Xmn*I PCR-RFLP[b]
01	–	AS-PCR[e]

CSN1S1 A* = A, G, I, H, 02; *CSN1S1* B2* = B2, L.
[a] Present work.
[b] Ramunno et al. (2000).
[c] Bevilacqua et al. (2002).
[d] Jansà Pérez et al. (1994).
[e] Cosenza et al. (2003).

by a high frequency of low (F, 0.368) and null (N, 0.227) alleles, which makes this population similar to goats of French origin (Ramunno et al., 1994). This characteristic may well be correlated to strong genetic erosion phenomena of the original population, as a consequence of crossbreeds with breeding stock belonging to Saanen, Maltese and Alpine breeds (Ciotola and Peretti, 2004).

4. Conclusion

Actually, the known goat *CSN1S1* alleles are associated with different expression levels (Chianese et al., 1997; Martin et al., 1999; Bevilacqua et al., 2002; Ramunno et al., 2005). It has been hypothesized that the observed differences in the expression of the goat *CSN1S1* gene could be the direct consequence of more elaborated systems of gene regulation (Ramunno et al., 2005). Indeed, comparative analysis of the *CSN1S1* (A, F and N alleles) gene promoters evidenced a G → A transition in position −1319 which seems to create an extra putative activator protein (AP-1) binding motif in the sequence of the F allele at a short distance from a constitutive link site of the same factor. The AP-1 transcriptional complexes are known to be important third messengers for target genes regulated by extracellular mediators; therefore it was considered possible that the goat *CSN1S1* gene would be responsive to AP-1 (Angel and Karin, 1991; Kerppola and Curran, 1993). A preliminary investigation upon goats with different known genotypes at the *CSN1S1 locus* and belonging to different breeds/genetic types, showed that the G → A substitution in −1319 position is present not only in the F allele but also in the remaining B-type alleles (Ramunno, L., pers. commun.). This suggests that the transcriptional activity of B-type alleles could be highlighted.

Until now *CSN1S1* B1, B2, B3, B4 and C alleles were genotyped by using milk electrophoresis as isoelectrofocusing (IEF). However, these methods are not useful for fast, unambiguous typing of individuals. Therefore, by using methods developed in this study, it is possible, both quickly and more accurately, to type animals independently of age, sex, and lactation and, for example, perform quail–quantitative analyses of mRNA (by RT-PCR and real-time PCR) in order to evaluate the different expression level of the single alleles and thus choose animals producing milk with particular chemical–physical and technological characteristics.

Acknowledgement

This is contribution no. 140 from the Department of Soil, Plant, Environment and Animal Production Sciences, Agriculture Faculty, University of Naples "Federico II".

References

Angel, P., Karin, M., 1991. The role of Jun, Fos and the AP-1 complex in cell—proliferation and transformation. Biochim. Biophys. Acta 1072, 129–157.

Bevilacqua, C., Ferranti, P., Garro, G., Veltri, C., Lagonigro, R., Leroux, C., Pietrolà, E., Addeo, F., Pilla, F., Chianese, L., Martin, P., 2002. Interallelic recombination is probably responsible for the occurrence of a new alpha(s1)-casein variant found in the goat species. Eur. J. Biochem. 269 (4), 1293–1303.

Brignon, G., Mahé, M.F., Ribadeau Dumas, B., Mercier, J.C., Grosclaude, F., 1990. Two of the three genetic variants of goat αs1-casein which are synthesized at a reduced level have an internal deletion possibly due to altered RNA splicing. Eur. J. Biochem. 193, 237–241.

Caroli, A., Chiatti, F., Chessa, S., Rignanese, D., Bolla, P., Pagnacco, G., 2006. Focusing on the goat casein gene complex. J. Dairy Sci. 89 (8), 3178–3187.

Chianese, L., Ferranti, P., Garro, G., Mauriello, R., Addeo, F., 1997. Occurrence of three novel alpha s1-casein variants in goat milk. Milk Protein Polymorphism FIL-IDF, Palmerston North, New Zealand, pp. 259–267.

Ciotola, F., Peretti, V., 2004. Razze zootecniche in pericolo di estinzione: la capra napoletana. Vita Campagna 9, 48.

Cosenza, G., Illario, R., Rando, A., Di Gregorio, P., Masina, P., Ramunno, L., 2003. Molecular characterization of the goat *CSN1S1*^{01} allele. J. Dairy Res. 70 (2), 237–240.

Enne, G., Feligini, M., Greppi, G.F., Iametti, S., Pagani, S., 1997. Gene frequencies of caprine αs1-casein polymorphism in dairy goats. In: IDF Seminar "Milk Protein Polymorphism II", Palmerston North, pp. 275–279.

Goossens, M., Kan, Y.W., 1981. DNA analysis in the diagnosis of hemoglobin disorders. Method Enzymol. 76, 805–817.

Grosclaude, F., Martin, P., 1997. Casein polymorphism in the goat. In: IDF Seminar "Milk Protein Polymorphism II", Palmerston North, pp. 241–253.

Jansà Pérez, M.J., Leroux, C., Sanchez Bonastre, A., Martin, P., 1994. Occurrence of a LINE sequence in the 3′UTR of the goat αs1-casein E encoding allele associated with a reduced protein synthesis level. Gene 147, 177–179.

Jordana, J., Amills, M., Diaz, E., Angulo, C., Serradilla, J.M., Sanchez, A., 1996. Gene frequencies of caprine αs1-casein polymorphism in Spanish goat breeds. Small Rumin. Res. 20, 215–221.

Kerppola, T.K., Curran, T., 1993. Selective DNA bending by a variety of bZIP proteins. Mol. Cell. Biol. 13, 5479–5489.

Leroux, C., Martin, P., 1996. The caprine αs1- and β-casein are 12-kb apart and convergently transcribed. Anim. Genet. 27 (Suppl. 2), 93.

Leroux, C., Mazure, N., Martin, P., 1992. Mutations away from splice site recognition sequences might *cis*-modulate alternative splicing of goat αs1-casein transcripts. Structural organization of the relevant gene. J. Biol. Chem. 267, 6147–6157.

Martin, P., Leroux, C., 1994. Characterization of a further αs1-casein variant generated by exon skipping. In: Proceedings of the 24th International Society of Animal Genetics Conference, E43, Prague, p. 88 (Abstract).

Martin, P., Ollivier-Bousquet, M., Grosclaude, F., 1999. Genetic polymorphism of caseins: a tool to investigate casein micelle organization. Int. Dairy J. 9, 163–170.

Ramunno, L., Cosenza, G., Pappalardo, M., Pastore, N., Gallo, D., Di Gregorio, P., Masina, P., 2000. Identification of the goat *CSN1S1*F allele by means of PCR-RFLP method. Anim. Genet. 31 (5), 342–343.

Ramunno, L., Cosenza, G., Rando, A., Pauciullo, A., Illario, R., Gallo, D., Di Berardino, D., Masina, P., 2005. Comparative analysis of gene sequence of goat *CSN1S1* F and N alleles and characterization of *CSN1S1* transcript variants in mammary gland. Gene 345 (2), 289–299.

Ramunno, L., Rando, A., Di Gregorio, P., Capogreco, B., Masina, P., 1994. Indagine di popolazione sui geni a effetto maggiore sul contenuto di caseina αsl e β nel latte di capra. Zoot. Nutr. Anim. 20, 107–111.

Rijnkels, M., 2002. Multispecies comparison of the casein gene *loci* and evolution of casein gene family. J. Mammary Gland Biol. Neoplasia 7, 327–345.