View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Institutional Research Information System University of Turin

IRIS AperTO

UNIVERSITÀ
DEGLI STUDI
DI TORINO

This is the author's final version of the contribution published as:

Cosenza G., A. Pauciullo, D. Gallo, L. Colimoro, A. D'Avino, Mancusi, L. Ramunno. Genotyping at the CSN1S1 locus by PCR-RFLP and AS-PCR in a Neapolitan goat population. Small Ruminant Research, 74 (1-3), 2008, 84-90

DOI:10.1016/j.smallrumres.2007.03.010

The publisher's version is available at:
https://www.sciencedirect.com/science/article/pii/S0921448807000995

When citing, please refer to the published version.

Link to this full text:
https://ac.els-cdn.com/S0921448807000995/1-s2.0-S0921448807000995-main.pdf?_tid=dd33b996-baaf-4f88-bedc-9c5b05b6f309&acdnat=1521322866_9a14c679c8916dec9c8059e08766507a

This full text was downloaded from iris-Aperto: https://iris.unito.it/

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

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

2 **goat population**

3

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

5

6 *Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali,*

7 *Università degli Studi di Napoli "Federico II", Napoli, Italy*

8

9

10

11 * Corresponding author.

12 Tel.: +39 0812539004;

13 fax: +39 0817762886.

14 *E-mail address:* ramunno@unina.it

15

16

17

18

19

20

21

22

1 **Abstract**

2 The goat *CSN1S1* gene has for many years been an excellent model for demonstrating
3 that most of the variability observed in the as1-casein content in goat's milk is due to the
4 presence of autosomal alleles at a single structural *locus*. Until now, about 17 alleles
5 associated to at least four levels of as1-casein expression in milk have been described at
6 the *CSN1S1 locus* in the domestic goat (*Capra hircus*). The great importance of goat as1-
7 casein polymorphism is due to its qualitative as well as quantitative implications. In the
8 present work five PCR protocols (PCR-RFLPs, AS-PCR) were set up for rapid
9 genotyping of B1, B2*, B3, B4 and C *CSN1S1* alleles, until now detectable only by milk
10 electrophoresis. Application of these protocols, together with previously described
11 methods to identify *CSN1S1* 01, E, M, F, N and A* (*CSN1S1* A, G, I, H) alleles, allow us to
12 define, at DNA level, the genetic structure of the autochthonous goat reared in the province
13 of Naples for the highest number of possible alleles at this *locus*. Monitoring of CSN1S1
14 variability in the Neapolitan goat population indicates a high frequency of low (F, 0.368)
15 and null (N, 0.227) alleles.

16

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

18

19

20

21

22

23

1 **1. Introduction**

2 Among Ca-sensitive caseins (as1, þ and as2), the as1 fraction is without doubt the most
3 extensively inves- tigated in goat. Such protein is codified by a single autosomal gene
4 (*CSN1S1*) mapped on chromosome 6 and clustered with genes of the other casein fractions
5 (*CSN1S2*, *CSN2* and *CSN3* for as2, þ and k, respec- tively) in a DNA stretch of about 250
6 kb (Leroux and Martin, 1996; Rijnkels, 2002).

7 So far, at least 16 alleles have been identified, which are associated with different levels
8 of as1-casein expres- sion in the milk. A first group of alleles (A, B1, B2, B3, B4, C, H,
9 L, M) are related to a normal content of as1-casein (about 3.5 g/l), whereas alleles I and
10 E are associated to an intermediate content (about 1.1 g/l), and alleles F and G are related
11 to a low level of as1-casein in the milk (about 0.45 g/l). Alleles *CSN1S1* N, 01 and 02 are
12 'null' alleles and have been associated with the appar- ent lack of as1-casein in milk
13 (Chianese et al., 1997; Martin et al., 1999; Bevilacqua et al., 2002; Ramunno et al., 2005).

14 Most of the mutational events responsible for the formation of such alleles have already
15 been identi- fied. The B1 allele is the original one from which two divergent lineages of
16 alleles originate: A-type (A, 01, 02, I, G, H) and B-type (B2, B3, B4, C, E, F, L) alleles
17 (Chianese et al., 1997; Grosclaude and Martin, 1997). It has been hypothesized that the
18 *CSN1S1* N and M alleles could be originated by an interallelic recombination event
19 between A- and B-type alleles (Bevilacqua et al., 2002; Ramunno et al., 2005). In par-
20 ticular, the alleles associated to a normal content of this casein fraction originated from
21 single nucleotide substitutions responsible for aminoacid substitutions (Chianese et al.,
22 1997; Martin et al., 1999; Bevilacqua et al., 2002). While the molecular event
23 characteriz- ing the I allele is unknown (Chianese et al., 1997), the E allele is characterized

by insertion of a DNA seg- ment (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 muta- tional event is a transition (G A) occurring in the 5Ј-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 dele- tion 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 as1 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 pop- ulation is characterized by the alternative skipping of exons 9–11 and is responsible as a consequence, for the synthesis of a form of as1 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 tran- scribed 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, occur- ring 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

1 about 8.5 kb starting from the 181st nucleotide of the intron 12, and including the last 7
2 exons of the gene (Cosenza et al., 2003), while a large insertion, so far uncharacterized, is
3 the mutational event responsible for the 02 allele (Martin et al., 1999). Furthermore, recent
4 analysis of the *CSN1S1* gene promoter showed a transition G A in position 1623.
5 This mutation, besides characterizing the N allele, would also characterize the 01 allele,
6 being polymorphic for the A allele. The latter result would suggest the existence of a second
7 A allele (called A2) from which the two null alle- les would originate (Ramunno et al.,
8 2005), supporting the idea that alleles 01 and 02 derive from two different A subtypes
9 (Grosclaude and Martin, 1997).
10 While the *CSN1S1* A and B* (B* = B1, B2, B3, B4, C) alleles appear more frequently in
11 the autochthonous goat population of southern Italy, alleles E and F are more frequent in
12 French, Swiss and Spanish breeds (Ramunno et al., 1994; Jordana et al., 1996; Enne et al.,
13 1997; Grosclaude and Martin, 1997; Caroli et al., 2006).
14 The aim of the present study is to establish the genetic structure at *CSN1S1 locus* of the
15 Neapolitan goat and to set up methods for the identification of *CSN1S1* B1, B2 B3, B4,
16 C alleles by using PCR-RFLP (PCR-restriction fragment polymorphism) and AS-PCR
17 (allele specific- PCR).
18
19 **2. Materials and methods**
20 2.1. *DNA samples*
21 The research was carried out on 285 individual DNA sam- ples of local Neapolitan goats
22 reared on different farms in the Sorrento Peninsula (southern Italy). Such population is
23 the result of the crossbreed of goat Neapolitan mainly with Saanen, Malta and Alpine

1 goats. The main attitude of the Neapoli- tan goat is the kids and milk production
2 (approximately 450 l for lactation) (Ciotola and Peretti, 2004). Genomic DNA was
3 extracted from blood leukocytes according to the method of Goossens and Kan (1981).
4
5 2.2. *Genotyping at goat CSN1S1 locus by PCR based methods*
6
7 Alleles *CSN1S1* M, E and F, N and 01 were detected by means of ACRS-PCR (amplified
8 created restriction site-PCR) (Bevilacqua et al., 2002), PCR (Jansà Pérez et al., 1994),
9 *Xmn*I PCR-RFLP (Ramunno et al., 2000) and AS-PCR (Cosenza et al., 2003),
10 respectively. Furthermore, based on the nucleotide sequence data, the PCR-RFLP
11 procedure developed for geno- typing of *CSN1S1* F and N alleles using *Xmn*I allows the
12 *CSN1S1* A* allele (*CSN1S1* A, G, I, H) to be discriminated (Ramunno et al., 2000).
13 In order to identify the goat carriers of *CSN1S1* C, B3, B2* (*CSN1S1* B2, L) and B1
14 alleles, genotyping methods based on PCR-RFLP were developed, whereas AS-PCR
15 was set up to detect the carriers of *CSN1S1* B4 allele. Primer sequences and the thermal
16 amplification conditions are reported in Tables 1 and 2, respectively. PCR was carried
17 out by using Gene Amp PCR System 2400 (Perkin-Elmer). The *CSN1S1* A, G, I, H
18 and *CSN1S1* B2 and L alleles were gathered in *CSN1S1* A* and *CSN1S1* B2* groups,
19 respectively, since they are indistinguishable with the methods developed in this study.
20 The 25 μl reaction mix for each PCR product comprised: 100 ng of genomic DNA, 50
21 mM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% Triton X-100, 3 mM $MgCl_2$, 5 pmol of each
22 primer, dNTPs each at 400 μM, 2.5 U of *Taq* DNA Polymerase (Promega, Madison, WI),
23 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 ampli- fied 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, includ- ing the 10th exon and part of the 11th exon of the goat *CSN1S1* gene, *CSN1S1* "non-B1" homozygous indi- viduals 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 ampli- fied 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 (CTNAG). *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 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

1 backwards from alleles B1 to C along the phylogenetic tree (Fig. 3). This is was necessary
2 since these alleles originated one by one in successive mutations.
3 The results of B-type allele genotyping (*CSN1S1* B1, B2*, B3, B4, C) by means of the
4 set up protocols confirmed the origin of each allele as reported in the phylogenetic tree
5 (Fig. 3).
6 Genotyping the whole population did not show car- riers of *CSN1S1* M and 01 allele, and
7 allowed the observation of 26 out of 45 possible genotypes with 9 alleles present in the
8 investigated population. The dis- tribution of the observed genotypes and the frequency of
9 alleles at *CSN1S1 locus* are reported in Table 4. In particular, among the strong alleles of
10 the B-type group (total frequency, 0.18), our research evidenced a higher frequency of B3
11 (0.072) and B4 (0.085) alleles. In com- parison to the other local populations reared in the
12 rest of southern Italy, local Neapolitan goats are characterized by a high frequency of low
13 (F, 0.368) and null (N, 0.227) alleles, which makes this population similar to goats of
14 French origin (Ramunno et al., 1994). This character- istic may well be correlated to
15 strong genetic erosion phenomena of the original population, as a consequence of
16 crossbreeds with breeding stock belonging to Saanen, Maltese and Alpine breeds (Ciotola
17 and Peretti, 2004).
18

19 **4. Conclusion**

20 Actually, the known goat *CSN1S1* alleles are associ- ated with different expression levels
21 (Chianese et al., 1997; Martin et al., 1999; Bevilacqua et al., 2002; Ramunno et al.,
22 2005). It has been hypothesized that the observed differences in the expression of the
23 goat *CSN1S1* gene could be the direct consequence of more elaborated systems of gene

1 regulation (Ramunno et al., 2005). Indeed, comparative analysis of the *CSN1S1* (A, F
2 and N alleles) gene promoters evidenced a G A transition in position 1319 which seems
3 to create an extra putative activator protein (AP-1) binding motif in the sequence of the
4 F allele at a short distance from a constitutive link site of the same factor. The AP-1
5 transcriptional complexes are known to be important third messengers for target genes
6 regulated by extra- cellular mediators; therefore it was considered possible that the goat
7 *CSN1S1* gene would be responsive to AP-1 (Angel and Karin, 1991; Kerppola and
8 Curran, 1993). A preliminary investigation upon goats with different known genotypes
9 at the *CSN1S1 locus* and belonging to different breeds/genetic types, showed that the G
10 A substitution in 1319 position is present not only in the F allele but also in the remaining
11 B-type alleles (Ramunno, L., pers. commun.). This suggests that the transcriptional
12 activity of B-type alleles could be highlighted.
13 Until now CSN1S1 B1, B2, B3, B4 and C alleles were genotyped by using milk
14 electrophoresis as isoelectrofocusing (IEF). However, these methods are not useful for fast,
15 unambiguous typing of individuals. Therefore, by using methods developed in this study,
16 it is possible, both quickly and more accurately, to type animals independently of age, sex,
17 and lactation and, for example, perform quail–quantitative analyses of mRNA (by RTPCR
18 and real-time PCR) in order to evaluate the different expression level of the single alleles
19 and thus choose animals producing milk with particular chemical–physical and
20 technological characteristics.
21
22 **Acknowledgement**
23

1 This is contribution no. 140 from the Department of Soil, Plant, Environment and
2 Animal Production Sciences, Agriculture Faculty, University of Naples “Federico II”.
3
4 **References**

5 Angel, P., Karin, M., 1991. The role of Jun, Fos and the AP-1 complex in cell—
6 proliferation and transformation. Biochim. Biophys. Acta 1072, 129–157.
7
8 Bevilacqua, C., Ferranti, P., Garro, G., Veltri, C., Lagonigro, R., Ler- oux, C., Pietrolà, E.,
9 Addeo, F., Pilla, F., Chianese, L., Martin, P., 2002. Interallelic recombination is probably
10 responsible for the occurrence of a new alpha(s1)-casein variant found in the goat species.
11 Eur. J. Biochem. 269 (4), 1293–1303.
12
13 Brignon, G., Mahé, M.F., Ribadeau Dumas, B., Mercier, J.C., Grosclaude, F., 1990.
14 Two of the three genetic variants of goat as1-casein which are synthesized at a reduced
15 level have an inter- nal deletion possibly due to altered RNA splicing. Eur. J. Biochem. 193,
16 237–241.
17
18 Caroli, A., Chiatti, F., Chessa, S., Rignanese, D., Bolla, P., Pagnacco, G., 2006. Focusing
19 on the goat casein gene complex. J. Dairy Sci. 89 (8), 3178–3187.
20
21 Chianese, L., Ferranti, P., Garro, G., Mauriello, R., Addeo, F., 1997. Occurrence of three
22 novel alpha s1-casein variants in goat milk. Milk Protein Polymorphism FIL-IDF,
23 Palmerston North, New Zealand, pp. 259–267.

1

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

4

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

7

8 Enne, G., Feligini, M., Greppi, G.F., Iametti, S., Pagani, S., 1997. Gene frequencies of
9 caprine as1-casein polymorphism in dairy goats. In: IDF Seminar "Milk Protein
10 Polymorphism II", Palmerston North, pp. 275–279.

11

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

14

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

17

18 Jansà Pérez, M.J., Leroux, C., Sanchez Bonastre, A., Martin, P., 1994. Occurrence of a
19 LINE sequence in the 3ʹUTR of the goat as1-casein E encoding allele associated with a
20 reduced protein synthesis level. Gene 147, 177–179.

21

22 Jordana, J., Amills, M., Diaz, E., Angulo, C., Serradilla, J.M., Sanchez, A., 1996. Gene
23 frequencies of caprine as1-casein polymorphism in Spanish goat breeds. Small Rumin.

1 Res. 20, 215–221.

2

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

5

6 Leroux, C., Martin, P., 1996. The caprine as1- and þ-casein are 12-kb apart and
7 convergently transcribed. Anim. Genet. 27 (Suppl. 2), 93.

8

9 Leroux, C., Mazure, N., Martin, P., 1992. Mutations away from splice site recognition
10 sequences might cis-modulate alternative splicing of goat as1-casein transcripts.
11 Structural organization of the relevant gene. J. Biol. Chem. 267, 6147–6157.

12

13 Martin, P., Leroux, C., 1994. Characterization of a further as1-casein variant generated
14 by exon skipping. In: Proceedings of the 24th International Society of Animal Genetics
15 Conference, E43, Prague, p. 88 (Abstract).

16

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

19

20 Ramunno, L., Cosenza, G., Pappalardo, M., Pastore, N., Gallo, D., Di Gregorio, P.,
21 Masina, P., 2000. Identification of the goat CSN1S1F allele by means of PCR-RFLP
22 method. Anim. Genet. 31 (5), 342–343.

23

1 Ramunno, L., Cosenza, G., Rando, A., Pauciullo, A., Illario, R., Gallo, D., Di Berardino,
2 D., Masina, P., 2005. Comparative analysis of gene sequence of goat CSN1S1 F and N
3 alleles and characterization of CSN1S1 transcript variants in mammary gland. Gene 345
4 (2), 289–299.
5
6 Ramunno, L., Rando, A., Di Gregorio, P., Capogreco, B., Masina, P., 1994. Indagine di
7 popolazione sui geni a effetto maggiore sul contenuto di caseina asl e þ nel latte di capra.
8 Zoot. Nutr. Anim. 20, 107–111.
9
10 Rijnkels, M., 2002. Multispecies comparison of the casein gene loci and evolution of
11 casein gene family. J. Mammary Gland Biol. Neoplasia 7, 327–345.
12
13
14

1 Table 1
2 Oligonucleotide primers, positions and restriction enzymes for PCR-RFLPs and AS-PCR
3

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]

4
5 [a] Restriction enzymes used for PCR-RFLP genotyping.
6 [b] Numbering of primers agrees with the nucleotide sequence of the goat *CSN1S1* gene
7 (EMBL Acc. No. AJ504710).
8
9

1 Table 2
2 Thermal amplification programs for (a) PCR and (b) AS-PCR Cycle
3

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

4
5

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.

1 Table 4
2 Number of individuals typed (no.), observed genotypes and allele frequencies at *CSN1S1*
3 *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).

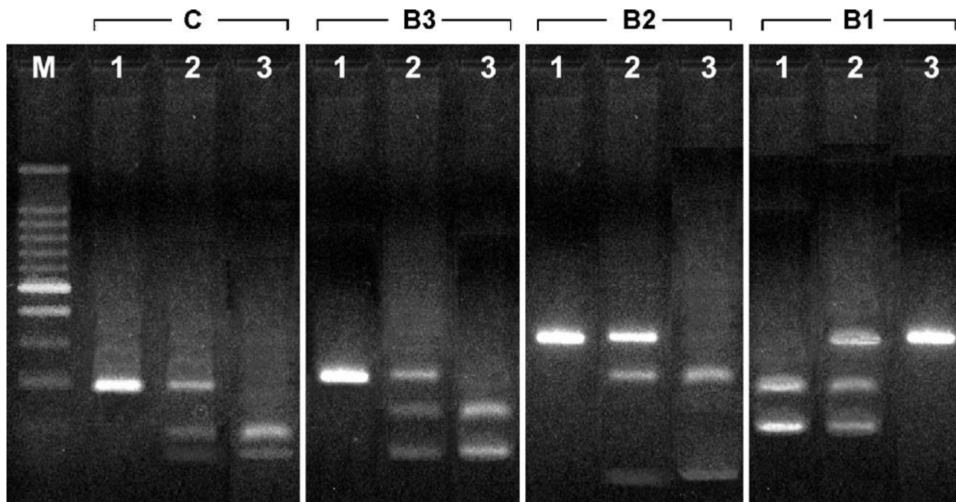
[c] Bevilacqua et al. (2002).

[d] Jansà Pérez et al. (1994).

[e] Cosenza et al. (2003).

4
5

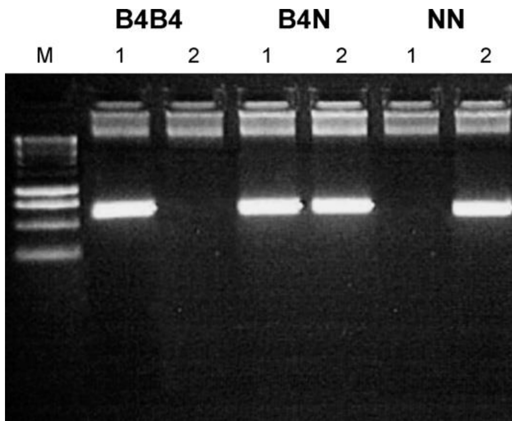
1



2

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

8



1

2 Fig. 2. Typing of *CSN1S1* B4 allele by AS-PCR. Lines 1 are amplicons obtained with a
3 specific forward primer for *CSN1S1* B4 allele, lines 2 are products obtained with a specific
4 forward primer for not *CSN1S1* B4 allele, M is a 100 bp molecular ladder (Promega).
5 *CSN1S1* N = "non- B4" allele.
6

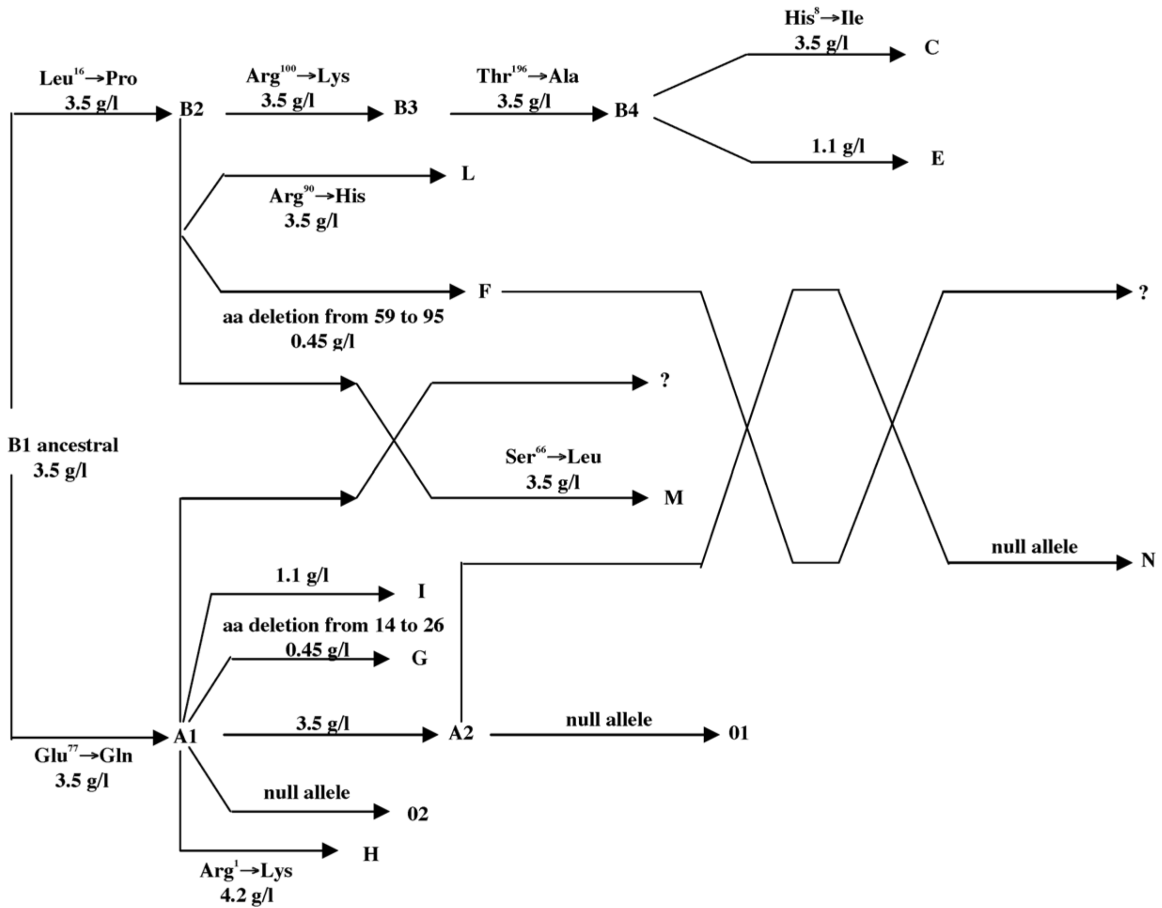


Fig. 3. Phylogeny tree for the goat *CSN1S1* alleles and differences between the corresponding variants. The new proposed phylogeny with respect to those proposed by Chianese et al. (1997) and Bevilacqua et al. (2002), has been enriched with two novel variants (N and A2) reported in Ramunno et al. (2005). The as1-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.