Identification of the goat *CSN1S1*^{F} allele by means of PCR-RFLP method

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The relative amounts of the four casein fractions (αs1, β, αs2 and κ) affect the physico-chemical, nutritional and technological properties of milk of domestic ruminants[1].

Polymorphism at the *CSN1S1 locus* in goat species is of interest because of its high degree of polymorphism and differences in the level of protein synthesis.

The *A*, *B* and *C* alleles are associated with a 'high' level of αs1-casein in milk (around 3.5 g/l), the *E* allele with a 'medium' level (1.1 g/l), the *F* and *G* alleles with a 'low' level (0.45 g/l), 0_1 and 0_2 alleles being 'null' alleles, without αs1-casein in the milk of homozygotes. Recently, the *B* allele has been divided into four alleles: B_1 (considered as the ancestor allelic form), B_2 (previously idenfied as *B*), B_3 and B_4 [2].

A single nucleotide deletion (C) at the 23rd nucleotide of the ninth exon and two insertions, one of 11 bp (CGTAATGTTTC), located 73 nucleotides downstream of the 5′ splice site of the ninth intron, and the other of 3 bp (AAT or TAA), interrupting the long polypyrimidine stretch upstream of the 3′ splice site of the ninth intron, were identified as mutations potentially responsible for the alternative skipping of exons 9, 10 and 11 of the goat *CSN1S1*^{F} allele[3].

Though the deletion inside the ninth exon was confirmed by means of polymerase chain reaction (PCR) amplification with allele specific primers[3], no method for identification of the insertions/deletions of the ninth intron has been proposed. The aim of the present study was to develop a method for a simultaneous typing for the deletion at the ninth exon and the 11-bp insertion in the downstream intron.

Milk and blood samples were obtained from 180 unrelated goats belonging to an undefined genetic type reared in Southern Italy. Milk samples were analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) at alkaline pH. DNA was estracted from leucocytes[4] obtained from blood samples collected with Na_2EDTA as anticoagulant.

All the DNA samples were analysed for the presence of *CSN1S1*^{F} and *CSN1S1*^{01} alleles by two Allele Specific-PCRs ([3]; Ramunno, personal communication) and the *CSN1S1*^{E} allele by PCR[5]. The region of the goat *CSN1S1* gene between nucleotides 208 and 420 (EMBL accession number X59835; [3]) spanning part of eigth intron, the ninth exon and part of the ninth intron was amplified and digested with *Xmn*I.

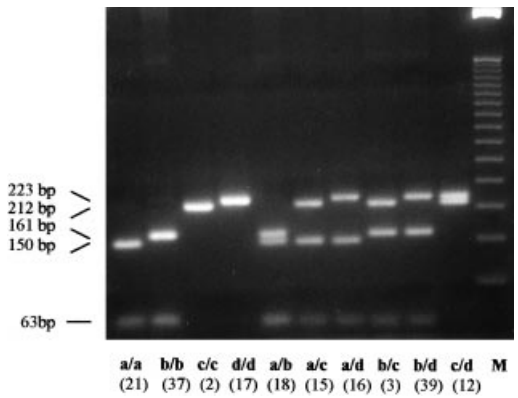

Fig. 1. Observed genotypes after *Xmn*I digestion of fragments obtained by means of PCR of the DNA region spanning from the eigth to the ninth intron of goat *CSN1S1* gene. D^+ = presence of cytosine deletion; D^- = absence of cytosine deletion; I^+ = presence of the 11 bp insertion; I^- = absence of the 11 bp insertion. *M* = marker (50 bp DNA ladder, Promega). The number of observed individuals is indicated in brackets.

Table 1. Observed allele and haplotype frequencies after *Xmn*I digestion of fragment obtained by means of PCR of the DNA region spanning from the eighth to the ninth intron of goat *CSN1S1* gene

Allele	Frequency	Haplotype	Frequency	RFLP (bp)
D^+	0.3750	$D+I^+$	0.2806	223
D^-	0.6250	$D+I^-$	0.0944	212
I^+	0.6528	$D-I^+$	0.3722	63+161
I^-	0.3472	$D-I^-$	0.2528	63+150

The PCR was carried out in a 50 μl reaction mixture containing: 100 ng of genomic DNA, 10 pmol of each primer (forward: 5′ TTCTAAAAGTCTCAGAGGCAG 3′, reverse: 5′ GGGTTGATAGCCTTGTATGT 3′), 1.25 U of *Taq* DNA polymerase (Promega, Italia, Milano, Italy), 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% Triton X-100, 3 mM $MgCl_2$, dNTPs each at 400 μM, 0.04% BSA.

The amplification protocol consisted: an initial cycle of 97 °C for 2 min, 60 °C for 45 s and 72 °C for 2 min 30 s; then 30 cycles of 94 °C for 45 s, 60 °C for 45 s, 72 °C for 2 min 30 s with a progressive increase of 4 s for each cycle in the extension step; with an extension step at 72 °C for 10 min in the final cycle. A total volume of 20 μl of each PCR product was digested with 10 U of *Xmn*I endonuclease for 5 h at 37 °C following the supplier's directions for buffer condition. PCR and digestion products were analysed by means of electrophoresis in 4% agarose gel stained with ethidium bromide.

The differences in length of the amplified fragments, determined by the 11 bp insertion, can be detected by means of the simple DNA electrophoresis (I^+, presence, 223 bp or 224 bp; I^-, absence, 212 bp or 213bp), whereas *Xmn*I restriction endonuclease digestion allows carriers of the single nucleotide deletion at ninth exon to be identified since the restriction site (GAANN/NNTTC) of the enzyme is altered (D^+, presence, D^-, absence). Therefore, by analysing the restriction fragment length polymorphism (RFLP) determined by *Xmn*I digestion, direct identification of haplotypes (D^+-I^+, 223 bp; D^+-I^-, 212 bp; D^--I^+, 63 bp + 161 bp; D^--I^-, 63 bp + 150 bp) (Fig. 1) determined by the two polymorphic sites is possible.

Frequencies of alleles at both *loci* as well as haplotype frequencies relative to the combinations of such alleles are reported in Table 1.

Comparison between results obtained from analyses on both DNA and milk samples of informative individuals revealed that haplotype D^--I^- is associated with *CSN1S1*^{A} and *CSN1S1*^{01} alleles, haplotype D^--I^+ is associated with with *CSN1S1*^{B} and *CSN1S1*^{E} alleles, haplotype D^+-I^+ is associated with with *CSN1S1*^{F} allele. No SDS-PAGE variant appears to be associated with the D^+-I^- haplotype. It is known that goat αs1 casein D and G variants comigrate with β-casein in SDS-PAGE[2,6]. However, at present, we do not know whether this haplotype is associated with an already known (O_2, D and G) or a new allele of the goat *CSN1S1 locus*. Nevertheless, data presented in this paper demonstrate that the insertion of 11 bp, located 73 nucleotides downstream the 5′ splice site of the ninth intron, does not affect, by itself, expression of the αs1 casein gene and that deletion of the 23rd nucleotide of the ninth exon is not specific of the *CSN1S1*^{F} allele. Furthermore, typing of both polymorphic sites is necessary in order to identify carriers of *CSN1S1*^{F} allele.

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Ovine microsatellite *OarKP6* isolated from a BAC containing the ovine interferon gamma gene

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Source/description: The microsatellite *OarKP6* was derived from a BAC containing the ovine interferon gamma (*IFNG*) gene. We confirmed the BAC contained *IFNG* exon 1 sequence, and the same BAC was physically mapped by Gill *et al.*[1]. The microsatellite was isolated using vectorette polymerase chain reaction (PCR)[2], and primers were designed from sequence flanking the AC repeat. The sequence of this locus has been submitted to GenBank (AF223411).

Primer sequences:
OarKP6 (GT strand): 5′-GCCCTGTGTCTCGTGTAACTCAC-3′
(CA strand): 5′-CCACAGGGTTGCAAAGAATCA-3′

PCR conditions: The PCR was carried out in a total volume of 10 μl, with the following constituents at the final concentration indicated in brackets: genomic DNA (approximately 100 ng); dNTPs (200 μM); tricine pH 8.8 (45 mM); $(NH_4)_2SO_4$ (11 mM); β-mercaptoethanol (6.7 mM); EDTA (4.5 μM); spermidine (0.25 mM); bovine serum albumin (200 μg/ml); $MgCl_2$ (4.5 mM); unlabelled primer (500 nM); ^{33}P-labelled primer (8 nM); *Taq* DNA polymerase (0.03 U). The GT primer was end labelled using [γ^{33}P] ATP (NEN) and T4 polynucleotide kinase (NEB). Thermocycling was performed on a Techne PHC-3 (Cambridge, UK) thermocycler using the following conditions: three cycles at 95 °C for 45 s, 60 °C for 1 min; three cycles at 95 °C for 45 s, 57 °C for 1 min; three cycles at 95 °C for 45 s, 54 °C for 1 min; three cycles at 95 °C for 45 s, 51 °C for 1 min; 20 cycles at 92 °C for 45 s, 48 °C for 1 min. No extension step was used.

Polymorphism and allele frequency: One hundred and seventy unrelated animals from five sheep breeds were genotyped with *OarKP6*, and seven different alleles were observed. The allele frequencies are listed in Table 1.

Mendelian inheritance: Codominant segregation of alleles was verified by genotyping *OarKP6* in the AgResearch International Mapping Flock[3] (IMF).

Table 1. Frequencies of *OarKP6* alleles in five sheep breeds

Allele	Awassi	Soay	Merino	Romney	Texel
193	0	0	0	0.05	0
195	0	0	0	0	0.14
197	0	0.01	0	0	0
199	0	0	0.01	0	0
201	1.0	0.95	0.52	0.77	0.72
203	0	0	0.04	0.13	0.09
205	0	0.04	0.43	0.05	0.05
(*n*)	47	47	46	19	11

Chromosomal location: Testing for linkage to all markers in the IMF database assigned *OarKP6* to ovine chromosome 3, close to the interferon gamma gene, as expected. We observed no recombination between *OarKP6*, an *IFNG* RFLP marker and a tetranucleotide microsatellite found in intron 1 of ovine *IFNG*[4]. Both of these markers had been previously mapped in this flock[5], but both have only two alleles. This note describes our attempt to discover a more informative marker in this region.

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Syntenic assignment of the selectin P gene (*SELP*) to bovine chromosome 16

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Sequence of PCR primers and PCR conditions: Primers were designed from the 3' untranslated region of the bovine cDNA sequence reported in GenBank (accession number L12041). PCR primers were as follows:

CD62P-F:	5′ CCAATCCCCACAGGATCCTATT 3′
CD62P-R:	5′ GTCTGCTCTGGTGCAGAACTAA 3′

A 146-bp fragment was amplified by PCR in a final volume of 10 μl containing 100 ng genomic DNA and 1·5 mM $MgCl_2$. Samples were preheated for 5 min at 94 °C, subjected to 35 cycles (94 °C, 15 s; 58 °C, 20 s; 72 °C, 20 s), and to a final extension step of 5 min at 72 °C in a MJ Research PTC 100 thermocycler, using Promega PCR kit (Promega, Madison, WI, USA).

Chromosomal assignment: Chromosomal assignment of the *SELP* gene was determined by PCR typing of the INRA hamster-bovine somatic hybrid cell panel using the primers CD62P-F and CD62P-R (Fig. 1). This panel consists of 38 cell lines and has been previously described by Laurent *et al.*[1] The gene was assigned to BTA16 with a correlation coefficient of 1 with *D16S20* (*BM6430*).[2] *SELP* has been mapped to mouse chromosome 1 (86·6 cM) and human chromosome 1q23-q25. These locations are in agreement with the comparative mapping data (human/cattle by heterologous painting obtained by Hayes;[3] human/mouse/cattle, mammalian homologies, MGD[4]). BTA16 corresponds with two different regions of human chromosome 1, p36-qter and q23-q42 and two different mouse chromosomes, MMU4 and MMU1. This new gene location contributes to the comparative gene maps among BTA16, HSA1 and MMU1.

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Erratum

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. *Animal Genetics* **31**, 342.

The publisher would like to apologise for the incomplete publication of the legend to Figure 1 in the above paper. The figure and the complete figure legend are now reproduced below.

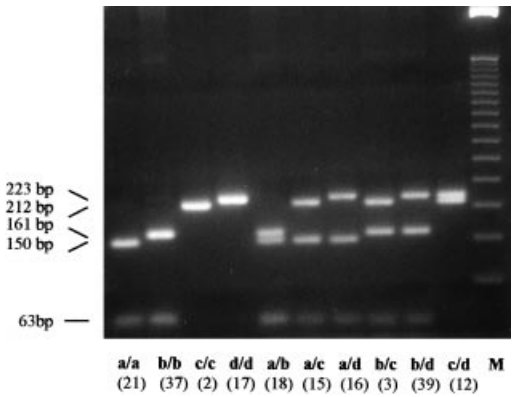

Figure 1 Observed genotypes after *Xmn*1 digestion of fragments obtained by means of PCR of the DNA region spanning from the eighth to the ninth intron of goat *CSN1S1* gene. a = D^{-} I^{-}; b = D^{-} I^{+}; c = D^{+} I^{-}; d = D^{+} I^{+}. D^{+} = presence of cytosine deletion: D^{-} = absence of cytosine deletion; I^{+} = presence of the 11 bp insertion; I^{-} = absence of the 11 bp insertion. *M* = marker (50 bp DNA ladder, Promega). The number of observed individuals is indicated in brackets.