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Molecular characterization of the goat *CSN1S1*^{01} allele

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Caseins (α_{s1}, β, α_{s2}, e κ) represent about 80% of the whole protein content of ruminant milk. Each of these proteins is encoded by single copy genes (*CSN1S1*, *CSN2*, *CSN1S2* and *CSN3*, respectively) clustered on a ~200-kb segment of chromosome 6 (Ferretti et al. 1990; Gallagher et al. 1994) in the order: *CSN1S1*, *CSN2*, *CSN1S2* and *CSN3* (Mercier & Vilotte, 1993). Furthermore, in cattle and goat *CSN1S1* and *CSN2* are convergently transcribed (Leroux & Martin, 1996; Rijnkles et al. 1997) and are only 20 and 12 kb apart, respectively.

Because of the strong similarity in the genomic structure, a common phylogenetic origin of genes of calcium-sensitive caseins (*CSN1S1*, *CSN2* and *CSN1S2*) has been hypothesized (Stewart et al. 1984; Groenen et al. 1993). In particular, *CSN2* is spread over 9·0 kb and consists of nine exons ranging in size from 24 (exon 5) to 492 bp (exon 7) (Roberts et al. 1992), while *CSN1S2* is about 18 kb long and is divided in to 18 exons ranging in size from 21 (exon 4) to 266 bp (exon 18) (Bouniol et al. 1994). *CSN1S1* is quite similar to *CSN1S2* since it is characterized by 19 exons ranging in size from 24 (exons 5, 6, 7, 8, 10, 13, 16) and 382/388 bp (exon 19) spread over 17·5 kb (Jansá Perez et al. 1994).

In recent years, alleles associated with strong differences in the level of expression, and that are consequently responsible for marked differences in the physicochemical properties of milk, have been identified in the goat calcium-sensitive casein genes. Each of these *loci* shows at least an allele associated with a 'null' content of the corresponding protein (Leroux et al. 1990; Ramunno et al. 1992, 2001; Mahè & Grosclaude, 1993). Such alleles are characterized by different mutations: single point mutations, responsible for premature stop codons, characterize null alleles of the *CSN2* (Rando et al. 1996; Persuy et al. 2000) and *CSN1S2* (Ramunno et al. 2001) *loci*; large DNA rearrangement (deletion/insertion) events of unknown origin and location characterize the two null alleles (*CSN1S1*^{01} and *CSN1S1*^{02}) of the *CSN1S1 locus* (Martin et al. 1999).

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Fast methods of identification of carriers of null alleles at both *CSN1S2* and *CSN2 loci* are available, whereas correct identification of carriers of the null alleles at the *CSN1S1 locus* can be obtained only by means of Southern blot (Di Gregorio et al. 1989; Leroux et al. 1990; Ramunno et al. 1991). The aim of the present work was to analyse the goat *CSN1S1*^{01} allele in order to identify the location of the deletion event and set up a fast and economic method of analysis, based on PCR, to identify carriers of the allele.

Materials and Methods

DNA samples

The research was carried out on blood samples obtained from 250 goats belonging to a local population reared in the province of Naples (Italy). DNA was extracted from leucocytes according to Goossens & Kan (1981).

Southern blot

DNA samples were analysed by means of Southern blot (Southern, 1975) using *Pst*I restriction endonuclease and a bovine α_{s1}-casein cDNA as probe (Stewart et al. 1984).

PCR analyses

Amplification of the DNA region between the 12th exon of the CSN1S1^{01} *allele and the 3′ of the* CSN2 *gene.* In order to amplify a long DNA region, a PCR reaction protocol was set up by using the TaKaRa LA Taq™ DNA polymerase (Takara Shuzo Co., Japan) and the following primers: $\alpha12^{F}$ 5′-AACGTGCCCCAGCTG-3′, nt 408–422 of EMBL accession number X59836 (Leroux et al. 1992) and $\beta9^{F}$ 5′-TCTTTGATGTTAAAAATAGCTGTGG-3′, nt 465–489 of EMBL accession number M90562 (Roberts et al. 1992). The 50-µl reaction mixture contained: 100 ng genomic DNA, 10 pmol each primer, 2·5 U LA Taq™ DNA polymerase (Takara), LA PCR Buffer II 1X (Takara), 2·5 mM $MgCl_2$, dNTPs each at 400 µM. The amplification

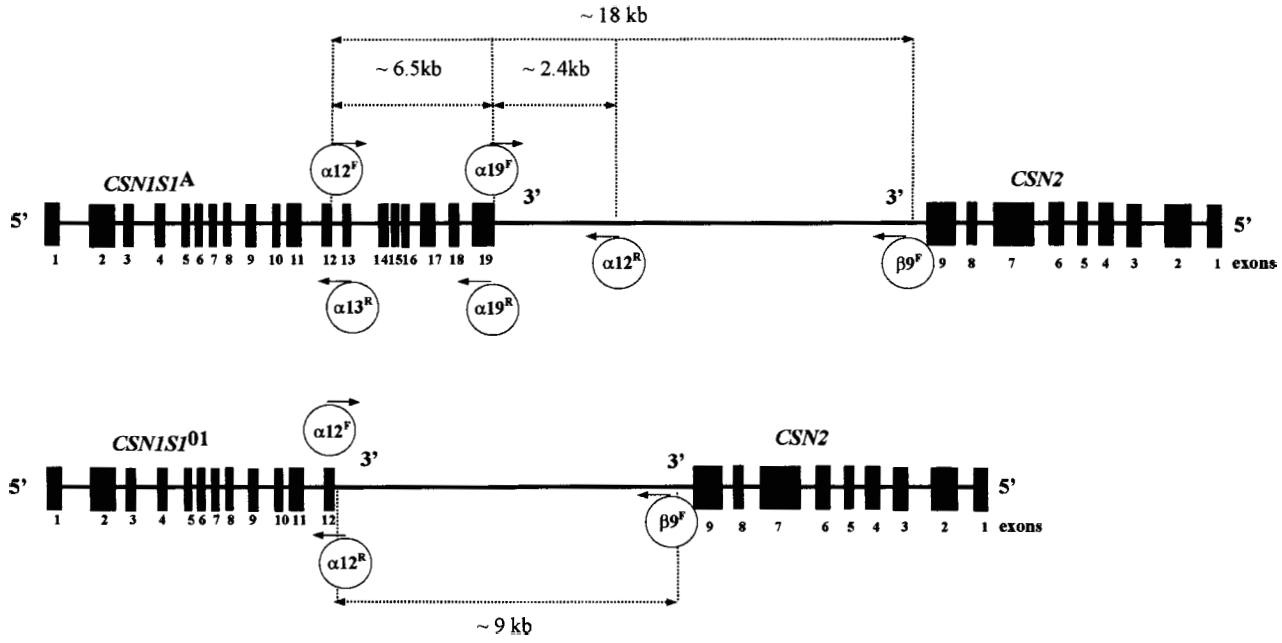

Fig. 1. Structure of the DNA regions containing the goat *CSN1S1* (A and 01 alleles) and *CSN2* genes and location of primers.

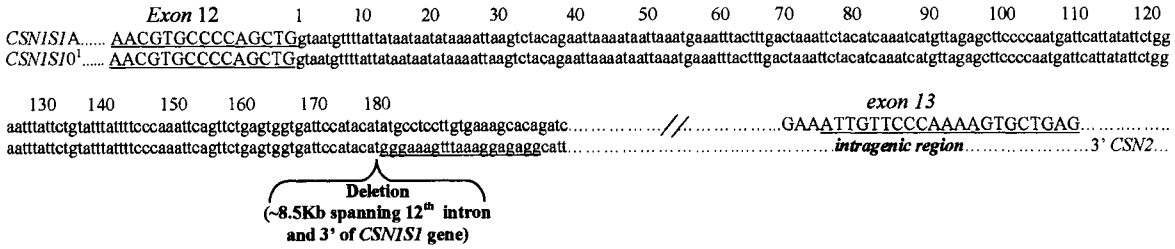

Fig. 2. Partial sequence of the 12th exon and 12th intron of the *CSN1S1*^{A} (EMBL acc.n° AJ252127) and *CSN1S2*^{01} (EMBL acc.n° AJ252126) alleles. AS-PCR primers are underlined.

protocol consisted of 31 cycles: the first cycle involved a denaturation step at 97 °C for 2 min, an annealing step at 59 °C for 45 s and an extension step at 72 °C for 10 min. The next 30 cycles were performed under the following conditions: 94 °C for 45 s, 59 °C for 45 s, 72 °C for 10 min with a progressive increase of 8 s for each cycle in the extension step. In cycle 31, conditions remained the same for denaturation and annealing steps but the extension step was carried out at 72 °C for 20 min.

Amplification of the DNA regions between the 12th and the 19th exon of the CSN1S1^{A} *allele and between the 19th exon and the first 20 nucleotides located at the 3′ end of the deletion.* Individual DNA samples from *CSN1S1*A/A goats were amplified by using the TaKaRa LA Taq™ DNA polymerase and the following primers: $\alpha12^{F}$ plus $\alpha19^{R}$ 5′-ATCTCAGTTACTGCACACAGT-3′, complementary to nt 1062–1082 of EMBL accession number X59836 (Leroux et al. 1992) and $\alpha19^{F}$ (complementary to $\alpha19^{R}$ oligonucleotide) plus $\alpha12^{R}$ 5′-CCTCTCCTTT-AAACTTTCCC-3′ (complementary to nt 190–209 of EMBL accession number AJ252126), respectively. The amplification protocol was as reported in the previous paragraph.

Amplification of the DNA region between the 12th and the 13th exon of the CSN1S1^{A} *allele.* PCR was accomplished using a Promega Taq DNA polymerase, the previously indicated $\alpha12^{F}$ oligonucleotide as forward primer and $\alpha13^{R}$ 5′-CTCAGCACTTTTGGGAACAAT-3′ oligonucleotide as reverse primer, complementary to nt 426–446 of EMBL accession number X59836 (Leroux et al. 1992). The 50-µl reaction mixture contained: 100 ng genomic DNA, 10 pmol each primer, 2·5 U Taq DNA polymerase (Promega), 50 mM KCl, 10 mM Tris-HCl (pH 9·0), 0·1% Triton X-100, 3 mM $MgCl_2$, dNTPs each at 400 µM, and 0·04% BSA. The amplification protocol consisted of 31 cycles: 94 °C for 45 s (first cycle 2 min at 97 °C), 59 °C for 45 s, 72 °C for 2 min 30 s (with a progressive

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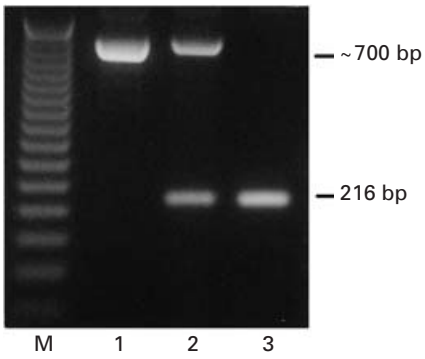

Fig. 3. DNA electrophoretic patterns of the amplified fragments characterizing the goat *CSN1S1*^{A} and *CSN1S1*^{01} alleles. M=Marker 50 bp (Promega); lane 1: *CSN1S1* A/A; lane 2: *CSN1S1* A/0^{1}; lane 3: *CSN1S1* 0^{1}/0^{1}.

increase of 4 s for each cycle and an extension step at 72 °C for 10 min in the last cycle).

Allele Specific-Polymerase Chain Reaction (*AS-PCR*). To the reaction mix for the amplification of the DNA region between the 12th and the 13th exon of the *CSN1S1* gene, 10 pmol of the $\alpha12^{R}$ oligonucleotide were added. Amplification products were analysed by means of electrophoresis on 1–2% agarose gels in TBE buffer. Location of primers indicated in Materials and Methods is shown in Fig. 1.

DNA sequencing

Nucleotide sequencing was carried out according to the dideoxynucleotide chain-termination technique (Sanger et al. 1977) using a BigDye™ Terminator cycle sequencing kit (Applied Biosystems, Warrington, UK) and an ABI PRISM 377-18 (Applied Biosystems, Foster City, CA, USA) nucleotide sequencer.

Results and Discussion

The population analysis obtained with Southern blots of 250 individual goat DNA samples showed one homozygote and ten heterozygotes for an allelic fragment of ~6·7 kb which, according to Ramunno et al. (1991), characterizes the large deletion specific for the *CSN1S1*^{01} allele.

PCR analyses of the *CSN1S1* gene of goats with different genotype at this *locus* showed no amplification products from the region located downstream of the 12th exon of the *CSN1S1*^{01}/^{01} individual. As a consequence, the deletion spanning the last seven exons of the gene starts in the 12th intron. Since milk of carriers of the *CSN1S1*^{01} allele is characterized by a normal amount of β-casein, we amplified the DNA region between the last exon (12th) of the *CSN1S1*^{01} allele and the 3′ side of the convergently transcribed *CSN2* gene. As a comparison, we amplified the DNA region between the 12th and the 13th exon of the *CSN1S1* gene of a *CSN1S1*^{A}/^{A} goat (Fig. 1).

Analysis of the electrophoretic patterns of PCR products indicated that the DNA region between the 12th exon of the *CSN1S1*^{01} allele and the 3′ of the *CSN2* gene is about 9·0 kb long and that the DNA region between the 12th and the 13th exons is about 700 bp long in *CSN1S1*$^{A/A}$ individuals.

Comparison of the DNA sequences obtained from fragments of 9·0 kb and 700 bp showed that the deletion characterizing the *CSN1S1*^{01} allele starts from nucleotide 181 of the 12th intron (EMBL accession number AJ252126) (Fig. 2). To obtain a more accurate estimate of the length of the deletion, the DNA regions between the 12th and the 19th exons and between the 19th exon and the first 20 nucleotides located downstream of the deletion were amplified in a *CSN1S1*$^{A/A}$ individual. The amplified fragments show a length of about 6·5 kb and 2·4 kb, respectively (Fig. 1). Consequently, the deletion characterizing the *CSN1S1*^{01} allele is at least 8·5 kb long and spans the last 7 exons and the 3′ region of the gene. Identification of the nucleotide sequence located downstream of the deletion allowed us to design a specific oligonucleotide for the *CSN1S1*^{01} allele and, therefore, to set up an Allele-Specific PCR (AS-PCR). Analysis of AS-PCR products showed that the amplified fragment characterizing the *CSN1S1*^{A} allele is about 700 bp spanning the DNA region between the 12th and the 13th exon whereas the amplified fragment characterizing the *CSN1S1*^{01} allele is 216 bp long and spans part of the 12th exon, the first 180 nucleotides of the 12th intron and the first 20 nucleotides located downstream of the deletion (Fig. 3). PCR conditions used in the present study do not allow amplification of the latter DNA fragment in individuals characterized by a normal gene where the distance between the two primers ($\alpha12^{F}$ and $\alpha12^{R}$) is about 9 kb.

Setting up of the AS-PCR reaction was necessary to reduce time and costs due to amplification of large DNA fragments. The results obtained by typing the 250 goat DNA samples with the AS-PCR method agree with results obtained by using the Southern blot method. Hence the AS-PCR method can be easily and affordably used for a fast and economic identification of carriers of the goat *CSN1S1*^{01} allele.

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