J. Dairy Sci. 88:1878–1881

Short Communication: Predominance of β-Casein (*CSN2*) *C* Allele in Goat Breeds Reared in Italy

S. Chessa,[1] E. Budelli,[2] F. Chiatti,[1] A. M. Cito,[3] P. Bolla,[1] and A. Caroli[3]
[1]Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, Università degli Studi di Milano, 20134 Milano, Italy
[2]Fondazione Parco Tecnologico Padano, Centro Ricerche e Studi Agroalimentari, 20090 Segrate (Milano), Italy
[3]Dipartimento di Sanità e Benessere Animale, Università di Bari, 70010 Valenzano (Bari), Italy

ABSTRACT

A protocol for the rapid and simultaneous genotyping of *A*, *C*, and *0′* CSN2 alleles in goat was developed by single strand conformational polymorphism polymerase chain reaction (SSCP-PCR) technique. Screening the CSN2 variability in 7 goat breeds reared in Italy validated the genotyping test. The SSCP-PCR technique was also suitable for monitoring CSN2 polymorphism. In particular, the discrimination between CSN2*A and CSN2*C is important because the 2 corresponding protein variants cannot be separated by standard typing techniques. The monitoring of CSN2 variability in the goat breeds indicates the predominance of the *C* allele. In most breeds, CSN2*C occurred with the highest frequency, except in Saanen where CSN2*A and CSN2*C showed similar frequencies. Variant CSN2*C occurred with a frequency of 0.68 (Camosciata), 0.70 (Jonica), 0.71 (Garganica), 0.82 (Maltese), 0.87 (Cilentana), and 0.97 (Orobica). The alignment among the mature CSN2 sequences of different species suggests that CSN2*A is the ancestral allele compared with CSN2*C. Interestingly, the CSN2*A goat variant showed higher frequencies in selected breeds (Saanen and Camosciata).
(**Key words:** goat, β-casein, genetic polymorphism, single strand conformational polymorphism)

Abbreviation key: CSN2 = β-casein, **IEF** = isoelectrofocusing, **SSCP** = single strand conformational polymorphism.

Genetic polymorphism of milk proteins has been intensively studied in goat because of the deep relationships with functional and biological properties affecting milk quality, composition, and technological characteristics (Martin 1993; Grosclaude et al., 1994). Goat caseins show a complex qualitative and quantitative variability, characterized by several genetic polymorphisms as well as by multiple post-translation modifications. Different transcriptional and post-transcriptional mechanisms control casein gene expression, dramatically affecting the technological properties of milk (Martin et al., 2002).

Casein genes are organized as a cluster including in the order α_{s1}-casein, β-casein (**CSN2**), α_{s2}-casein, and κ-casein loci (Ferretti et al., 1990; Threadgill and Womack, 1990). Within the cluster, the first 2 casein loci are only 12 kb apart and convergently transcribed (Leroux and Martin, 1996). In goats, the entire casein gene cluster region spans about 250 kb on chromosome 6 (Hayes et al., 1993; Popescu et al., 1996).

As far as CSN2 is concerned, 3 protein variants were found to be associated with a normal β-casein content: A, B, and C. The B variant was detected by isoelectrofocusing (**IEF**), resulting in some bands markedly closer to the gel cathodic position than in the A variant (Mahé and Grosclaude, 1993). Genetic control of the B allele was supported by the segregation analysis in one available family. However, the variant was not further characterized. The C variant was identified by peptide mass fingerprinting and tandem mass spectrophotometry (Neveu et al., 2002). This variant differs from the A in the mono amino acid substitution Ala_{177} to Val_{177} of the mature protein. Because both amino acids are neutral, the mutation is not detectable by screening protein techniques such as milk IEF. At the DNA level, the protein polymorphism is justified by a nucleotide substitution GCA (Ala_{177}) → GTA (Val_{177}), as can be observed by alignment of the sequence GenBank accession number AF409096 (Wang et al., 2001; direct submission) with AH001195 sequence (Roberts et al., 1992).

Furthermore, 2 different null CSN2 alleles were identified, both characterized by mutations responsible for premature stop codons in exon 7, one in southern Italian breeds (Ramunno et al., 1995; GenBank accession number AJ011019) and the other in the Creole and Py-

Received October 19, 2004.
Accepted December 20, 2004.
Corresponding author: Anna Caroli; e-mail: am.caroli@veterinaria.uniba.it.

renean goat (Persuy et al., 1999; GenBank accession number AF172260). The mRNA analysis revealed that the transcript product amounts were almost 10 (Ramunno et al., 1995) and 100 (Persuy et al., 1999) times lower for the null alleles than for the A variant. As far as the nomenclature is concerned, the 2 null alleles were differentiated respectively by *0* (AF172260) and *0′* (AJ172260) by Neveu et al. (2002). This nomenclature will be used in the present communication.

To obtain further information on goat CSN2 variability, exon 7 was analyzed by single strand conformational polymorphism- (**SSCP-**) PCR in 7 Italian goat breeds. An SSCP-PCR protocol was developed for typing CSN2**0′* as an alternative to allele-specific PCR (Ramunno et al., 1995). Different SSCP patterns were detected and sequenced. Moreover, milk samples were typed by IEF to check the correspondence between the DNA variation and the phenotypic expression.

Blood and individual milk samples were randomly collected from 4 Southern Italian goat breeds (Garganica, Jonica, Cilentana, and Maltese), 1 Northern Italian breed (Orobica), and 2 composite breeds (Camosciata and Saanen). Four hundred seventy-three samples were analyzed. Reference samples for the CSN2**0′* allele (GenBank accession number AJ011019) were also used. A commercial kit (GFXTM Genomic Blood DNA Purification kit, Amersham Biosciences, Piscataway, NJ) was used for DNA extraction from blood or milk.

A 374-bp fragment containing exon 7 of the goat CSN2 gene was amplified by PCR performed in a 25-μL reaction mixture containing 2 μL of DNA solution (100 to 150 ng), 10 pmol of each primer, and 1× PCR Master Mix (Fermentas, Vilnius, Lithuania). Primers were 5′CCC AAA GTG AAG GAG ACT ATG 3′, and 5′ CAT CAG AAG TTA AAC AGC ACA G 3′. The following conditions were used: an initial denaturation step of 95°C for 2 min, followed by 30 cycles of 94°C for 45 s, 58°C for 45 s, 72°C for 2 min, and a final extension step of 72°C for 5 min using a PTC-0200 DNA Engine thermal cycler (MJ Research Inc., Waltham, MA).

For SSCP, 6 μL of PCR product was added to 8 μL of denaturation solution (0.05% of xylene-cyanol, 0.05% of bromophenol blue, 0.02 *M* EDTA in deionized formamide). After heat denaturation at 95°C for 8 min, the samples were immediately chilled on ice and then run overnight (16 h) on 10% acrylamide:bisacrylamide gels (29:1) with 1.5% glycerol in 0.5× Tris-borate-EDTA buffer at 280 V and 12°C (Penguin Dual Gel Water-Cooled Electrophoresis System, OWL Scientific Inc., Woburn, MA). Bands were visualized by silver staining (Bassam et al., 1991).

The DNA samples showing different patterns on SSCP gels were randomly selected for sequencing. Eight samples were sequenced. Primers used for se-

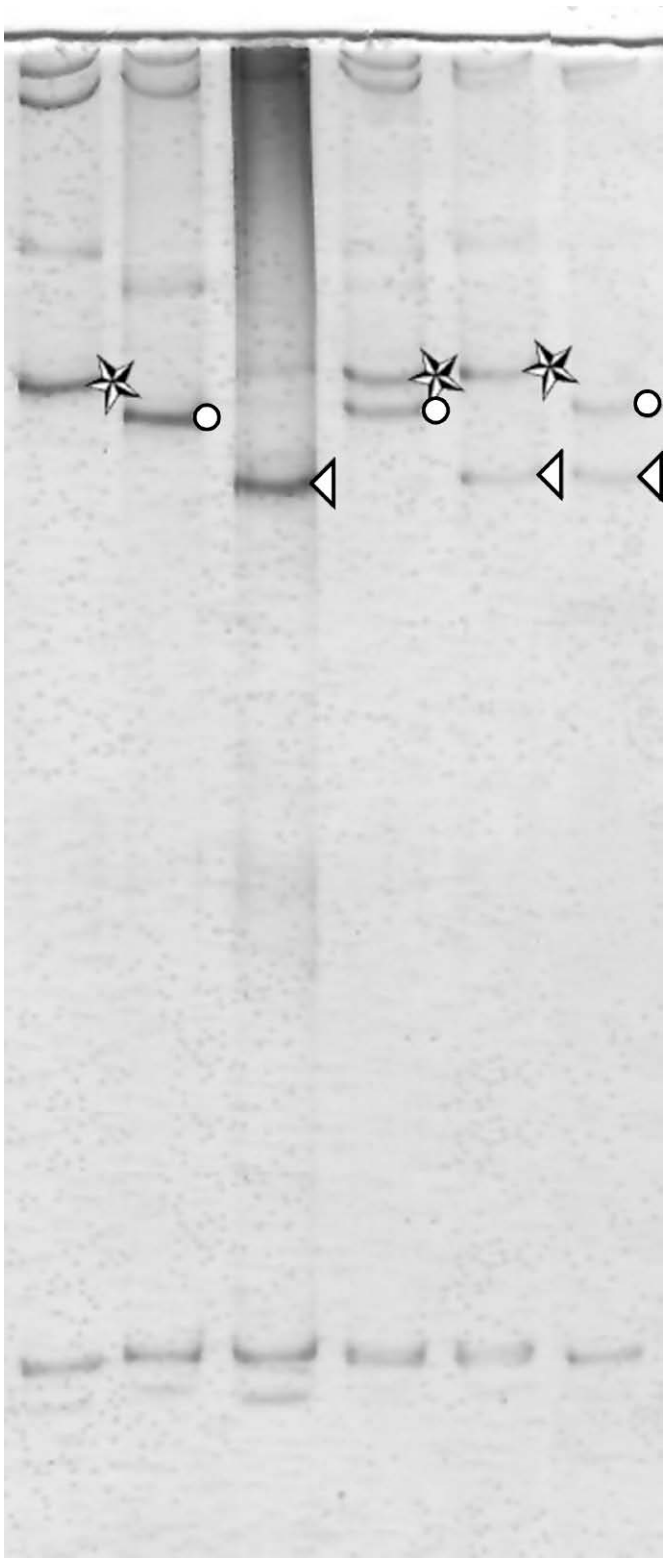

Figure 1. Single strand conformational polymorphism-PCR typing for the simultaneous detection of CSN2**A*, CSN2**C*, and CSN2**0′* (GenBank Accession number AJ011019). Genotypes are indicated for each sample. The discriminant band for each allele is also shown (star: CSN2**C*, circle: CSN2**A*; triangle: CSN2**0′*).