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An SNP in the goat *CSN2* promoter region is associated with the absence of β-casein in milk

G. Cosenza*, A. Pauciullo*, L. Colimoro*, A. Mancusi*, A. Rando†, D. Di Berardino* and L. Ramunno*

*Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Facoltà di Agraria, Università degli Studi di Napoli 'Federico II', Portici (Na) 80055, Italy. †Dipartimento di Scienze delle Produzioni Animali, Facoltà di Agraria, Università degli Studi della Basilicata, Potenza 85100, Italy

Summary

So far, at least eight alleles in the goat *CSN2* locus have been associated with the level of β-casein expression in milk. Alleles $CSN2^{A}$, $CSN2^{A1}$, $CSN2^{B}$, $CSN2^{C}$, $CSN2^{D}$ and $CSN2^{E}$ have been associated with normal content (allele effects of about 5 g of β-casein per litre), whereas the $CSN2^{O}$ and $CSN2^{O1}$ alleles have been associated with non-detectable levels of β-casein. Most of these alleles have been characterized genetically. Herein, we report the identification of a previously unreported SNP in the goat *CSN2* promoter region (AJ011018:g.1311T>C), which is associated with the absence of β-casein in the milk. Furthermore, we developed a PCR-based method that allows detection of this mutation.

Keywords β-casein, goat, null allele, promoter, SNP.

β-casein is the most abundant protein component (about 10 g/l) in goat milk (Grosclaude *et al.* 1987). The goat *β-casein* (*CSN2*) gene maps to chromosome 6 and its organization is similar to that observed in other species (Rijnkels 2002). To date, eight alleles identified at this locus are associated with two expression levels: the $CSN2^{A}$, $CSN2^{A1}$, $CSN2^{B}$, $CSN2^{C}$, $CSN2^{D}$ and $CSN2^{E}$ alleles are associated with normal content (Roberts *et al.* 1992; Mahé & Grosclaude 1993; Neveu *et al.* 2002; Galliano *et al.* 2004; Cosenza *et al.* 2005; Caroli *et al.* 2006), whereas the $CSN2^{O}$ and $CSN2^{O1}$ alleles are associated with non-detectable amounts of this protein (Ramunno *et al.* 1995; Persuy *et al.* 1999).

Analysis of the coagulation properties of milk with and without β-casein demonstrates that milk without β-casein shows longer coagulation times (about three times the normal value, 15–25 min vs. 4–7 min) and low curd firmness (so low that it is impossible to measure). Furthermore, cheese yield (caciotta at 30 days ageing) from milk without β-casein is about 80% of that obtained from milk with a normal content of this casein fraction (Chianese *et al.* 1993).

Address for correspondence

L. Ramunno, Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Facoltà di Agraria, Università degli Studi di Napoli 'Federico II', Portici (Na) 80055, Italy.
E-mail: ramunno@unina.it

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With the exception of the $CSN2^{B}$ allele, the DNA and protein sequences of these alleles are known (Roberts *et al.* 1992; Mahé & Grosclaude 1993; Ramunno *et al.* 1995; Persuy *et al.* 1999; Neveu *et al.* 2002; Galliano *et al.* 2004; Cosenza *et al.* 2005; Caroli *et al.* 2006). The $CSN2^{O1}$ allele is characterized by a single-nucleotide substitution at position 373 of the seventh exon (Ramunno *et al.* 1995) (AJ011018:g.8915C>T), while the $CSN2^{O}$ has a single-nucleotide deletion (adenine) in a row of four adenines between nucleotide 16 and 19 of exon 7 (Persuy *et al.* 1999) (AJ011018:g.8561delA). These mutations result in a truncated protein at amino acids 57 (Persuy *et al.* 1999) (p.Ile49SerfsX10) and 181 (Ramunno *et al.* 1995) (p.Gln182X) respectively.

Levels of mRNA product transcribed by the $CSN2^{O}$ and $CSN2^{O1}$ alleles are about 100 (Persuy *et al.* 1999) and 10 (Ramunno *et al.* 1995) times lower than normal expression respectively. More recently, Cunsolo *et al.* (2005) reported on the identification and characterization of a truncated β-casein in the milk of goats homozygous for the $CSN2^{O1}$ allele. The truncated protein contained 1–166 amino acid residues of the mature β-casein variant A.

So far, research on the polymorphisms associated with differences in the expression of β-casein has been mainly limited to exonic regions of *CSN2*. However, mutations in the promoter region might be responsible for differences in the level of gene expression by modifying either the level of transcription or the mRNA stability and, consequently, the

content of a particular protein in the milk (Martin *et al.* 2002; Prinzenberg *et al.* 2003; Szymanowska *et al.* 2004; Kuss *et al.* 2005). Moreover, it has been suggested that differential expression of various milk-protein alleles is a possible result of linkage between variants of coding and regulatory regions of their genes (van Eenennaam & Medrano 1991; Ramunno *et al.* 2005). Therefore, the objective of this study was to examine associations between polymorphisms in the 5′-flanking region of the goat *CSN2* gene and the β-casein content in the milk.

Comparisons between sequences of goat $CSN2^{A}$ (AJ011018) and $CSN2^{01}$ (AJ011019) alleles showed two transitions located in the 5′ gene-flanking region: g.1538A>G, which was already reported by Pappalardo *et al.* (1997), and g.1311T>C.

Because nucleotides T in position 1311 and A in position 1538 have been found not only in *Capra hircus* promoter sequences (DQ673920, DQ673919, AY834229, AY398686, M90559, AY311384) but also in other ruminant species such as *Ovis aries* (X79703), *Bubalus bubalis* (AY352050), *Bos taurus* (AJ973327, U47012, M55158, U47013, X14711, M75888) and *Bos grunniens* (AF194986), these sequences might represent the ancestral state of the gene.

To assess the effect of these two mutations in the promoter region on β-casein level in goat milk, we analysed the genomic DNA of 854 goats in five breeds: 74 Garganica, 115 Malta, 90 Alpine, 95 Saanen and 480 Southern Italy population of undefined genetic type. Genomic DNA was extracted from leucocytes (Goossens & Kan 1981) obtained from blood samples collected using Na_2EDTA as anticoagulant. Milk from the same individuals was previously analysed by SDS-PAGE according to Grosclaude *et al.* (1987). Moreover, each DNA sample was genotyped for the presence of the mutation g.8915C>T by allele-specific amplification (AS-PCR) (Ramunno *et al.* 1995). Genotyping for the transition g.1538A>G was performed by MseI PCR-RFLP (Pappalardo *et al.* 1997) and an AS-PCR was set up for the transition g.1311T>C.

Sequence primers used for the AS-PCR for the transition g.1311T>C (184 bp in length) and amplification conditions are listed in Table S1. Primers for the amplification of goat *CSN2* exon 9 were also included in the PCR reactions as a positive control (360 bp in length). Amplifications were performed in a 50-μl volume containing from 100 to 200 ng of genomic DNA, 1x PCR Buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.04% BSA, 10 pmol of each primer, 2.5 U Taq DNA polymerase (Promega). Amplified fragments were analysed by means of electrophoresis in 2% agarose gels stained with ethidium bromide.

In the present study, the lack of β-casein in the milk was always in *cis* with the g.1538G allele, but this allele has been associated with normal β-casein levels in other studies. Therefore, this mutation is believed not to be involved in the lack of expression of *CSN2*.

On the contrary, all goats whose milk produced an SDS-PAGE pattern apparently without the β-casein fraction (Fig. 1, lane 1) (15 Southern Italy and three Malta samples) and those goats with a band less intense than normal (about 50%, Fig. 1, lane 2) (11 Garganica, 15 Malta and 81 Southern Italy animals) were homozygous or heterozygous respectively for the g.1311C and g.8915T alleles (Table 1). All samples that produced a normal SDS-PAGE pattern (Fig. 1, lane 3) (63 Garganica, 97 Malta, 90 Alpine, 95 Saanen, 384 Southern Italy samples) were homozygous for the g.1311T and g.8915C alleles (Table 1). The frequencies of the g.1311C and g.8915T alleles were 0.074, 0.091 and 0.115 for the Garganica and Malta breeds and Southern Italy population respectively. The overall frequencies of g.1311C and g.1538G were 0.084 and 0.152 respectively.

Sequence analyses of the *CSN2* promoter region based on TRANSFAC 7.0 database (http://www.gene-regulation.com/pub/databases.html) indicate that the presence of g.1311C does not alter or create any regulatory sites (Fig. 2). However, given the absence of the β-casein in animals with this allele, it is possible that this mutation is involved in gene regulation processes. It is also possible this allele is in linkage disequilibrium with a causative mutation for lack of expression. Sequencing of the complete genomic sequence of the *CSN2* gene in these animals is needed.

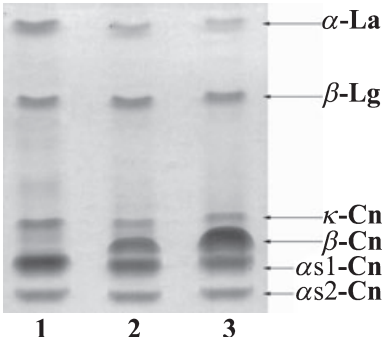

Figure 1 Electrophoretic pattern in SDS-PAGE of individual goat samples. Lane 1, $CSN2^{01/01}$; lane 2, $CSN2^{A*/01}$ and lane 3, $CSN2^{A*/A*}$, where A* = $CSN2^{A}$, $CSN2^{A1}$, $CSN2^{B}$, $CSN2^{C}$, $CSN2^{D}$ or $CSN2^{E}$ (indistinguishable by SDS-PAGE).

Table 1 Distribution of genotypes at the goat *CSN2* locus.

	g.1311T>C			g.8915C>T			g.1538A>G			
SDS-PAGE	TT	TC	CC	CC	CT	TT	AA	AG	GG	Total
$CSN2^{A*/A*}$	729	–	–	729	–	–	648	66	15	729
$CSN2^{A*/0}$	–	107	–	–	107	–	–	86	21	107
$CSN2^{0/0}$	–	–	18	–	–	18	–	–	18	18
Total	729	107	18	729	107	18	648	152	54	854

A*, alleles associated with a normal content of β-casein ($CSN2^{A}$, $CSN2^{A1}$, $CSN2^{B}$, $CSN2^{C}$, $CSN2^{D}$ and $CSN2^{E}$).

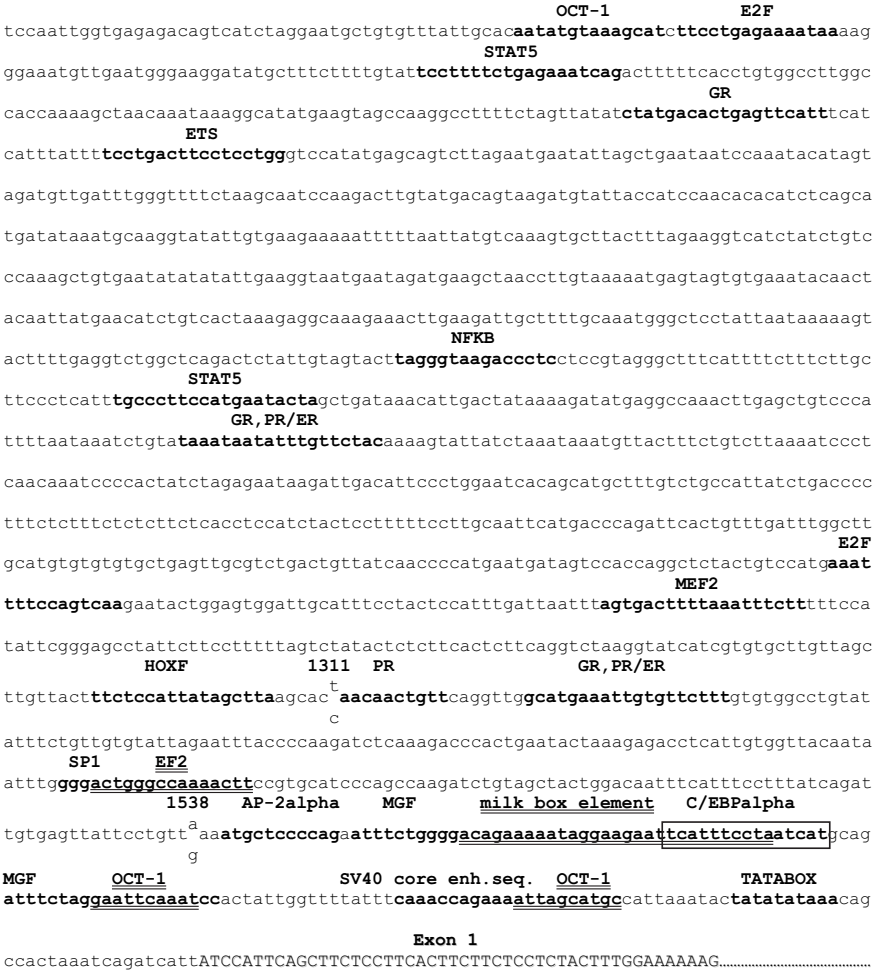

Figure 2 Nucleotide sequence of the 5′ flanking region and partial exon 1 of goat *CSN2* gene (numbering is according to AJ011018). Congruent and putative factors are double underlined, bold letters, or boxed. Transcription factor abbreviations: C/EBP, CCAAT/enhancer-binding protein; OCT-1, nuclear factor octamer-1; PR, progesterone receptor; MEF2, myocyte-specific enhancer-binding factor; EF2, E2F-myc activator/cell cycle regulator; SP1, GC-box factors; STAT5, signal transducer and activator of transcription 5; MGF, mammary gland factor recognition sequence; AP-2, alpha, activating enhancer binding protein 2 alpha; GR, glucocorticoid response element; NFKB, nuclear factor kappa B; HOXF, factors with moderate activity to homeo domain consensus sequence, ETS, E26 transformation-specific family of transcription factors.

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Supplementary material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2007.01649.x

Table S1 Primer sequences and amplification conditions for PCR.

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