SHORT COMMUNICATION

Genetic polymorphism of the K-casein (CSN3) gene in goats reared in Southern Italy

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ABSTRACT

K-casein (K-CN) represents one of the most important proteins determining the manufacturing properties of milk, because of its essential role in micelle formation and stabilisation.

Several genetic variants of K-CN have been described in goats. To investigate the occurrence of seven alleles and their distribution among breeds, a total of 170 animals, from six different breeds reared in Italy (Cilentana Nera, Derivata di Siria, Maltese, Jonica, Garganica and Cashmere), have been analysed in this paper by the primer extension method. Alleles A and B were found to be the most represented in all the analysed breeds; allele D is present only in Maltese and Cashmere animals with a very low frequency; while allele G has been found in all but two (Garganica and Cashmere) breeds. Alleles C, E and F were not present in the material used for this study.

Key Words: Genetic polymorphism, Goat, K-casein.

RIASSUNTO

POLIMORFISMI GENETICI DEL GENE DELLA K-CASEINA IN CAPRE ALLEVATE IN ITALIA DEL SUD

La K-caseina (K-CN) rappresenta una delle più importanti proteine che determinano le proprietà tecnologico-casearie del latte, a causa del suo ruolo essenziale nella formazione e stabilizzazione della micella. Numerose varianti genetiche della K-CN sono state descritte in capra. Al fine di investigare la presenza di sette alleli e la loro distribuzione in diverse razze, in questo lavoro sono stati analizzati 170 animali provenienti da sei razze allevate in Italia (Cilentana Nera, Derivata di Siria, Maltese, Jonica, Garganica e Cashmere) utilizzando il metodo primer extension. Gli alleli A e B sono risultati presenti con la frequenza più alta in tutte le razze analizzate; l’allele D è stato riscontrato solo negli animali di razza Maltese e Cashmere, con una frequenza molto bassa, l’allele G è stato trovato in tutte le razze in studio eccetto che nella Garganica e nella Cashmere. Gli alleli C, E e F sono invece risultati assenti negli animali usati in questo studio.

Parole chiave: Polimorfismo genetico, Capra, K-caseina.
Introduction

The polymorphism of milk proteins was described for the first time by Aschaffenburg and Drewry (1955). Since then, many studies have been performed to investigate milk protein polymorphism and great progress has been made with the introduction of analytical techniques that offer a greater degree of resolution, especially since the advent of DNA analysis methods. This has led to an extensive investigation of genetic polymorphism of the six main milk proteins and to the identification of several variants (reviewed by Ng-Kwai-Hang et al., 1998 and Martin et al., 2002).

A great interest concerned the influence of genetic variants on milk quantity, composition and technological properties (Grosclaude et al., 1994; Mariani and Summer, 1999).

K-CN, which constitutes about 15% of the total caseins, represents one of the most important proteins that determine the manufacturing properties of milk, because of its essential role in micelle formation and stabilisation (Swaisgood, 1993; Gutiérrez et al., 1996).

Several variants of this protein have been described in cows (reviewed in Kaminski, 1996), whereas K-CN is considered to be monomorphic in sheep (Moioi et al., 1998). In goats, different studies have analysed the polymorphism of the K-CN. At the protein level many variants were observed (Addeo et al., 1978; Russo et al. 1986; Di Luccia et al. 1990; Law and Tziboula, 1993), and different genetic variants have also been described (Yahyaoui et al., 2001; Caroli et al., 2001; Angiolillo et al., 2002; Yahyaoui et al., 2003; Chessa et al., 2003; Jann et al., 2004). Yahyaoui et al. (2001) detected, in French and Spanish breeds, seven polymorphic sites that resulted in three different alleles called A, B and C. In the same year two alleles (called A and B), differing at the protein level by isoelectrofocusing, were detected by Caroli et al. (2001) in German and Italian breeds. A new polymorphic position in exon four seems characteristic for the E allele, which until now was found exclusively in the autochthonous Italian breed Montefalcone (Angiolillo et al. 2002). Recently Yahyaoui et al. (2003) described two other alleles (F and G) in Italian breeds and wild goats, and a new method for the rapid and simultaneous genotyping of all the seven known K-CN variants, based on primer extension analysis. In the same year a PCR-SSCP method for the simultaneous identification of five K-CN alleles was also described (Chessa et al. 2003). During the drafting of this paper, additional K-CN variants were reported (Jann et al., 2004).

The simultaneous publication of different papers describing new K-CN alleles has led to a confusion in the literature, caused by the assignation of the same letter to different variants (Caroli et al., 2001; Yahyaoui et al., 2001; Chessa et al., 2003, Yahyaoui et al., 2003; Jann et al., 2004).

In the present study the method described by Yahyaoui et al. (2003) was used to investigate the occurrence of seven K-CN alleles in six goat breeds reared in the south of Italy. Five of them are autochthonous (Cilentana Nera, Derivata di Siria, Maltese, Jonica, Garganica), while the Cashmere goats belong to a breed recently introduced in the Basilicata region. The nomenclature proposed by Yahyaoui et al. (2003) has been adopted.

Material and methods

A total of 174 goats (Jonica = 13; Derivata di Siria = 39; Maltese = 29; Garganica = 45; Cilentana Nera = 32; Cashmere = 16) were analysed; they were randomly chosen, closely related animals were avoided.

DNA was isolated from blood following standard protocols (Ausubel et al., 1987). A 645-bp fragment, which contains all the exon 4 of the caprine K-CN, was amplified by PCR and screened to verify the distribution of the seven alleles (A-G) of the gene.

The PCR was performed as reported by Yahyaoui et al. (2003) using the primers:

I3F (5’-TCCCAATGTTGTACTTTCTTAACATC-3’)
Kb2 (5’-GCGTTGTCCTCTTTGATGTCTCCTAG-3’).

For a rapid and low cost genotyping, mutations were analysed by the primer extension analysis method, using the Multiplex SNAPSHOT ddNTP kit (PE Applied Biosystems, Foster City, CA), as
described by Yahyaoui et al. (2003). This technique detects single nucleotide polymorphisms (SNPs) based on the dideoxy single nucleotide extension of an unlabelled primer. The AmpliTaq DNA Polymerase adds a single fluorescently labelled ddNTP to the 3' end of the primer, designed on the template just before the mutation. Primers allow size- and colour-discrimination between the different alleles and were optimised to be used simultaneously (Yahyaoui et al., 2003).

Results and discussion

To avoid any confusion that might arise from the actual conflicting nomenclature, Table 1 reports the variants of the K-CN analysed. The nucleotides present at polymorphic positions and the corresponding amino acid changes in each variant are indicated.

Table 1. Variants of the caprine K-casein gene.

| Position (Exon 4) | Position (Protein) | A       | B       | C       | D       | E       | F       | G       |
|------------------|--------------------|---------|---------|---------|---------|---------|---------|---------|
| 104              | 44                 | A (Gln) | A (Gln) | A (Gln) | G (Arg) | A (Gln) | A (Gln) | A (Gln) |
| 166              | 65                 | G (Val) | G (Val) | A (Ile) | A (Ile) | G (Val) | G (Val) | A (Ile) |
| 242              | 90                 | A (Asp) | A (Asp) | A (Asp) | A (Asp) | G (Gly) | A (Asp) | A (Asp) |
| 328              | 119                | G (Val) | A (Ile) | A (Ile) | A (Ile) | A (Ile) | A (Ile) | A (Ile) |
| 440              | 156                | C (Ala) | C (Ala) | T (Val) | C (Ala) | C (Ala) | C (Ala) | C (Ala) |
| 448              | 159                | T (Ser) | T (Ser) | C (Pro) | C (Pro) | T (Ser) | C (Pro) | C (Pro) |

Nucleotides present at polymorphic positions and corresponding amino acid changes in each variant are indicated. Positions of nucleotides are relative to the start of exon 4.

Table 2. Frequencies of the k-casein variants in the analysed goat breeds.

| Breed             | n.   | A       | B       | C       | D       | E       | F       | G       |
|-------------------|------|---------|---------|---------|---------|---------|---------|---------|
| Maltese           | 29   | 0.086   | 0.879   | -       | 0.015   | -       | -       | 0.015   |
| Derivata di Siria | 39   | 0.076   | 0.897   | -       | -       | -       | -       | 0.025   |
| Jonica            | 13   | 0.250   | 0.583   | -       | -       | -       | -       | 0.166   |
| Garganica         | 45   | 0.077   | 0.922   | -       | -       | -       | -       | -       |
| Cilentana Nera    | 32   | 0.203   | 0.781   | -       | -       | -       | -       | 0.019   |
| Cashmere          | 16   | 0.035   | 0.9333  | -       | 0.035   | -       | -       | -       |
in this study. This is probably due to the small number of goats (13 and 45, respectively) considered for the breeds and the low frequency of the allele. On the contrary, the D allele has been detected in two animals of the Maltese breed and in one Cashmere goat.

As far as the E allele is concerned (Angiollilo et al., 2002), it is absent in all analysed breeds and is present exclusively in the Montefalcone goat. Therefore, this allele is likely to be used as a breed-specific marker for product identification.

Allele F, considered to be the ancestral type of caprine K-CN (Yahyaoui et al., 2003) resulted absent in all the breeds, whereas allele G, probably derived from a mutation of the F (Yahyaoui et al., 2003), is well represented, especially in the Jonica population.

The potential use of milk protein polymorphisms in selection programmes is very important, although more research should be done in order to attain greater knowledge of the mechanisms controlling their role and the level at which they can carry out their action.

In the meantime, the identification of different mutations is of pivotal importance in evaluating the level of genetic variability (both in local and standardised goat breeds) to be used as a reservoir once the effects of the single genetic variants, or haplotypes, on milk quality and cheesemaking properties are comprised.

Conclusions

The goat population analysed in this work completes the frame of the presence and distribution of known K-CN alleles in breeds reared in Southern Italy, most of which are of local origin. Rare and autochthonous goat breeds usually contain a variety of alleles, corresponding to genes with a potential effect on milk property and specificity. Since the quality of food is a promising option for animal production, it is essential to investigate and preserve animal genetic variability in rare and local breeds.

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