Molecular characterisation of κ-casein gene in Girgentana dairy goat breed and identification of two new alleles

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Abstract

The κ-casein fraction plays an important role in the formation, stabilisation and aggregation on casein micelles and thus affects technological and nutritional properties of milk. In this study, exon 4 of κ-casein (CSN3) gene was sequenced and analysed in Girgentana goat breed. Analyses of the obtained sequences showed the presence of A, B, D, and G known alleles and two new genetic variants, named D‘ and N. The new D‘ allele differs from D in one transition, G284→A284, which did not cause amino acid change. The new N allele differs from A in five single nucleotide polymorphisms (SNPs): T269/C269, G284/A284, G309/A309, G471/A471 and T591/C591, while it differs from C in one transition, i.e. T331→C331. Comparing the amino acid sequences of N and A alleles, the first two SNPs caused no amino acid change, whereas the other SNPs produced changes (Val65/Ile65, SNPs caused no amino acid change, whereas sequences of Val119/Ile119, and Ser159/Pro159, respectively).

Over the last years this breed has become almost extinct, in part as a consequence of the marked decrease in fresh goat milk consumption. The aim of this work was to investigate the genetic polymorphisms of CSN3 gene in the Girgentana dairy goat breed in order to assess genotypes distribution and to use this information in future conservation programmes for this breed considering that genotype could influence milk properties.

Materials and methods

Sampling and DNA extraction

A total of 205 individuals, all females, of Girgentana goat breed were randomly chosen. They belonged to 15 different herds located in different areas of Sicily. Samples were collected from buffy coats of nucleated cells using a salt-anticoagulant. Genomic DNA was extracted using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), samples were diluted to a final concentration of 50 ng/µL in ultrapure water and stored at 4°C until use.

Introduction

In the milk of ruminants, more than 95% of proteins are synthesised by six structural genes, four caseins (αs1-, β-, κs2- and κ-casein (CSN3)) and two whey proteins (αs1-lactalbumin and β-lactoglobulin). Polymorphisms of the four casein genes have been the focus of considerable research effort because of their potential effects on milk quality. The κ-casein fraction plays an important role in the formation, stabilisation and aggregation of the casein micelles and thus affects the technological (Mariani et al., 1976; Aleandri et al.; 1990; Lodes et al., 1996; Falaki et al., 1997) as well as nutritional properties of milk (Mercier et al., 1973, 1976; Malkoski et al., 2001).

The κ-casein gene comprises five exons (Coll et al., 1993, 1995) with the mRNA coding region for mature protein (171 amino acids) spanning from exon 3 (9 amino acids) to exon 4 (162 amino acids) (Yahayaoui et al., 2003). The CSN3 gene is considered to be monomorphic in sheep (Moioli et al., 1998) whereas several studies on goat CSN3 showed that this gene is highly polymorphic (Caroli et al., 2001; Yahayaoui et al., 2001; Angiolillo et al., 2002; Chessa et al., 2003; Yahayaoui et al., 2003; Jann et al., 2004; Reale et al., 2005; Prinzenberg et al., 2005; Gupta et al., 2009; Kiplagat et al., 2010). According to the last nomenclature proposed by Prinzenberg et al. (2005), a total of 16 DNA variants have been identified in the domestic goat, of which 13 are protein variants (named in alphabetical order from A to M) and 3 are silent mutations (B′, B″ and C′) involving a total of 15 polymorphic sites. Recently, Gupta et al. (2009) in Jakhrama goat breed and Kiplagat et al. (2010) in indigenous Eastern African goat population reported the presence of new genetic polymorphisms at CSN3 gene. These studies reported conflicting results for allele nomenclature because according to Prinzenberg et al. (2005) only missense mutations associated with amino acid changes should be indicated with new allele names (i.e. new letter), while silent mutations should be named with the same letter as the related protein-associated allele followed by prime symbol. The Girgentana goat is an ancient Sicilian goat breed reared in Southern Italy for its good dairy production. Average milk production was 224±66 L in the first lactation, and 320±109 L for later lactations (AIA, 2013). Due to sanitary policies, population size of Girgentana goat breed decreased of almost 90% in 20 years. In 1983, the population consisted of 30,000 individuals but, nowadays, only 374 heads are enrolled in the Herd Book (ASSONAPA, 2013).

Key words: CSN3 gene, Polymorphisms, New alleles, Girgentana goat breed.

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DNA amplification and purification

A 552 bp fragment of *Girgentana* goat κ-casein exon 4 (GenBank Acc. No. X60763) mRNA goat CSN3 was amplified by polymerase chain reaction (PCR) using the following primers: forward -AGAAATAATACCATTCTG- and reverse -TCTTTGTAGTCCTCTTATAGAG. The PCR reaction was performed in a 25 μL of final volume containing 1 μM of each primer, 1 mM of dNTP Mix, 1 U of Taq DNA polymerase (Fermentas, Hanover, MD, USA), 1X PCR buffer with KCl, 1.25 mM MgCl₂, and approximately 100-150 ng of genomic DNA. Thermal cycling conditions were 94°C for 90 sec for initial denaturation, 30 cycles of 45 sec each at 94°C, 50°C and 72°C, with a final extension at 72°C for 5 min. The PCR products were checked by electrophoresis on 2% agarose gel stained with SYBR Safe (Invitrogen, Carlsbad, CA, USA).

DNA sequencing reaction

All the collected samples were amplified and the PCR products purified in order to sequence and determine the complete nucleotide sequences. Polymerase chain reaction products were purified using 10 U of Exonuclease I and 1 U of Shrimp Alkaline Phosphatase (Fermentas). DNA sequencing reaction was carried out using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) with 5 μM of the same primers used in the PCR reaction. Cycle sequencing reaction was performed according to manufacturer’s instruction following Ethanol/EDET/Sodium Acetate precipitation. Sequencing analyses were performed in an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems).

Sequence data analysis

Nucleotide sequences obtained were checked using Sequencing Analysis Software v5.3.1 (Applied Biosystems) and subsequently analysed with SeqScape v2.5 Software (Applied Biosystems). Polymorphic sites were confirmed by visual examination of the electropherograms. Multiple alignments of the sequences were performed using ClustalW software (Thompson et al., 1994). The translation of DNA sequences to amino acid sequences was performed using ExPASy-Translate tool. The same software was used to calculate the isoelectric point (IP) of the new genetic variants found in *Girgentana* goat breed. The exact P value associated with the null hypothesis of Hardy-Weinberg equilibrium (HWE) was estimated using GENEPOP version 4.0.11 (Rousset, 2008). The programme performed a probability test using a Markov Chain.
No. AY027868) in one transition G284 allele differing from new according to Prinzenberg alleles identified in T591 /C591 ), while differing from breed (T583 Acc. No. AY350425) allele in one transition (Leu56/Leu56). The new cies at reported by Gupta Table 1. Considering the conflicting results sites detected in our samples, are showed in phisms (SNPs) described by Prinzenberg JX889424). All single nucleotide polymor- D, and alleles were identified only in two Nalleles belong to AIEF group (Table 1), which were found in our samples. The most common genotype was Girgentana goat samples. The most common genotypic classes were found in our Girgentana goat breed. The most common genotype was AB (39.5%) followed by AA (19.5%), AD (12.7%), and BB (11.7%). The other genotypes showed a frequency of less than 10% (Table 2). In this study, we found no homozygous D’D’, GG, and NN subjects. Caravaca et al. (2009), in a study on the effect of CSN3 genotypes on goat milk composition, showed that AB and BB genotypes were significantly associated with higher levels of total casein and protein content compared with the AA genotype, thus underlining the importance of taking into account the CSN3 genotype when performing selection for milk composition in dairy goats.

Results and discussion

Identified alleles in Girgentana goat breed

Sequencing analysis and alignment of the obtained sequences of CSN3 exon 4 showed the presence in Girgentana goat breed of A, B, D, and G known alleles and two new genetic variants (GenBank Acc. No. JX889419-JX889424). All single nucleotide polymorphisms (SNPs) described by Prinzenberg et al. (2005), including the two new polymorphic sites detected in our samples, are shown in Table 1. Considering the conflicting results reported by Gupta et al. (2009) and Kiplagat et al. (2010), we named D’ and N the two new alleles identified in Girgentana goat breed according to Prinzenberg et al. (2005). The new CSN3 D’ (GenBank Acc. No. JX889422) allele differing from CSN3 D (GenBank Acc. No. AY207668) in one transition G284→A284, which did not cause amino acid change (Leu56/Leu56). The new CSN3 N (GenBank Acc. No. JX889424) allele differing from CSN3 A (GenBank Acc. No. X00763) allele in five SNPs (T245/C245, G284/A284, G309/A309, G471/A471, and T530/C530), while differing from C (GenBank Acc. No. AY350425) allele in one transition (T383→C383). Comparing the amino acid sequences of CSN3 N and A alleles, the first two SNPs (T245/C245 and G284/A284) caused no amino acid change, whereas the other SNPs produced changes: Val149/Leu149, Val151/Leu151, and Ser159/Pro159, respectively. Comparison of CSN3 N allele with CSN3 C allele revealed the amino acid change Val156→Ala156.

Allele frequencies and genetic variability

Table 2 shows genotype and allele frequencies at CSN3 locus in Girgentana goat breed. The most frequent allele was A (0.480) followed by B (0.363), D (0.112), and N (0.034). The D’ and G alleles were identified only in two animals and in heterozygous conditions with a very low frequency (0.005). These results are not in agreement with those reported for Girgentana goat breed by Gigli et al. (2008), and by other authors for Italian (Sacchi et al., 2005), European and African (Prinzenberg et al., 2005) goat breeds, where the most frequent allele was B. Prinzenberg et al. (2005), proposed to differentiate the nomenclature at protein level from the one used at DNA level introducing two codes (AIP and BIP) corresponding to two IP's (IP=5.53 and 5.78, respectively) identified using isoelectrofocusing (IEF) method (Table 1). According to this nomenclature, among the CSN3 alleles found in our study, only the D and D’ alleles are included in BIP group, whereas A, B, G and N alleles belong to AIP group (Table 1), which represents the less favourable variants group in terms of milk composition and technological properties (Chiatti et al., 2007).

Nine genotypic classes were found in our Girgentana goat samples. The most common genotype was AB (39.5%) followed by AA (19.5%), AD (12.7%), and BB (11.7%). The other genotypes showed a frequency of less than 10% (Table 2). In this study, we found no homozygous D’D’, GG, and NN subjects. Caravaca et al. (2009), in a study on the effect of CSN3 genotypes on goat milk composition, showed that AB and BB genotypes were significantly associated with higher levels of total casein and protein content compared with the AA genotype, thus underlining the importance of taking into account the CSN3 genotype when performing selection for milk composition in dairy goats.

Observed and expected heterozygosity, fixation index F_s and P value associated with the null hypothesis of HWE were estimated. Significant departure from HWE was observed for Girgentana goat breed at CSN3 locus (P<0.05), probably due to heterozygote excess (Ho=0.6766 vs He=0.6243). This hypothesis could be confirmed considering Ho heterozygosity and F_s (-0.8655) values.

Conclusions

Two new genetic variants have been identified and characterised in Girgentana goat breed. Currently, phenotypic data are not available for this goat breed; hence, further studies could establish the possible association and the effects of polymorphisms on quantitative and qualitative characteristics of milk. Moreover, it could be useful to take into account CSN3 gene to use lines of goats producing different types of milk for specific cheese-making technologies or nutritional human needs.

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