



## SHORT COMMUNICATION

# Genetic characterisation of *CSN2* gene in *Girgentana* goat breed

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## Abstract

Among calcium sensitive caseins,  $\beta$ -casein is the most abundant in goat milk, representing up to 50% of total casein content. The goat  $\beta$ -casein locus has been widely investigated and at least ten alleles have been identified in different goat breeds. The aim of this work was to investigate the polymorphisms of  $\beta$ -casein gene in *Girgentana* dairy goat breed in order to assess the genotype distribution and evaluate how frequencies have changed during the last 10 years, as genotype is known to influence technological and nutritional milk properties. Sequencing analysis and alignment of the obtained sequences of  $\beta$ -casein exon 7, showed the presence of C, C1, and A strong alleles, and 0' null allele, with frequencies of 0.597, 0.326, 0.023, and 0.054, respectively. Seven genotypic classes were found in *Girgentana* goat breed and the most frequent genotype was CC1 (0.423) followed by CC (0.326), C1C1 (0.110), and C0' (0.096). No AA nor 0'0' homozygous individuals were found. The presence of strong alleles at *CSN2* gene in *Girgentana* goat breed could be useful for the production of milk with high protein content and good cheese-making properties. Moreover, food business operators should consider the possibility of reviving interest in *Girgentana* goat milk using weak and null genotypes at *CSN2* locus to make peculiar food products, such as drinking milk.

## Introduction

Caseins are the most abundant proteins in milk of ruminants and represent about 80% of total milk proteins, while the remaining part are whey proteins (mainly  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin, although other whey proteins such as immunoglobulins and lactoferrin are also present). It is well known that caseins are

encoded by four linked genes which form a unique cluster including  $\alpha$ S1-casein (*CNS1S1*),  $\beta$ -casein (*CNS2*),  $\alpha$ S2-casein (*CNS1S2*) and  $\kappa$ -caseins (*CNS3*) genes (Grosclaude *et al.*, 1978; Ferretti *et al.*, 1990; Rijnkels, 2002). Goat casein genes are mapped on chromosome 6 within a region that spans about 250 Kb (Hayes *et al.*, 1993; Popescu *et al.*, 1996). Among caseins, the  $\beta$ -casein is the most abundant in milk, representing up to 50% of total casein content. The goat *CSN2* encoding gene consists of nine exons ranging in size from 24 (exon 5) to 492 bp (exon 7) (Roberts *et al.*, 1992; Hayes *et al.*, 1993). At least, ten alleles have been identified in goat *CSN2* gene. In particular, seven of these alleles (A, A1, C, C1, E, 0, and 0') were characterised at DNA level (Rando *et al.*, 1996; Persuy *et al.*, 1999; Chessa *et al.*, 2005, 2008; Cosenza *et al.*, 2005), whereas B and D alleles were described only at protein level (Mahé and Grosclaude, 1993; Galliano *et al.*, 2004). Another variant has been found by Chianese *et al.* (2007) at protein level but it was not yet characterised. Furthermore, the genetic variants A, A1, B, C, C1, D, and E are associated with a normal  $\beta$ -casein content in milk (about 5 g/L per allele) (Roberts *et al.*, 1992; Mahé and Grosclaude, 1993; Neveu *et al.*, 2002; Galliano *et al.*, 2004; Cosenza *et al.*, 2005; Caroli *et al.*, 2006) while the two null alleles (0 and 0'), are associated with a non-detectable amount of this protein (Ramunno *et al.*, 1995; Persuy *et al.*, 1999).

The *Girgentana* goat is a Sicilian goat breed reared for its good dairy production. Due to sanitary policies, the size of the *Girgentana* goat breed decreased of almost 90% in 20 years, and nowadays, only 374 heads are reared in Sicily (ASSONAPA, 2013).

The aim of this work was to investigate the genetic polymorphisms of the  $\beta$ -casein gene in the *Girgentana* dairy goat breed in order to assess the genotype distribution, as it is known that genotype influences milk properties.

## Materials and methods

### Samples collection

A total of 196 samples, all females, of *Girgentana* goat breed were collected. The animals belonged to 10 different herds located in Sicily, among Agrigento and Palermo provinces. Samples were collected from 15 to 25 unrelated individuals per herd. About 10 mL of blood was used for genomic DNA extraction with a salting out method (Miller *et al.*, 1988). After extraction, the DNA samples were quan-

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tified, using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), then they were diluted to a final concentration of 50 ng/ $\mu$ L in ultrapure water and stored at 4°C until use.

### Amplification protocols

Different polymerase chain reaction (PCR) protocols were used to genotype A, A1, C, C1, E, and 0' alleles of goat *CSN2* gene in *Girgentana* goat breed. The 0 allele was not genotyped because it has been identified only in Creole and Pyrenean goat breeds (Persuy *et al.*, 1999). The first protocol was used to amplify a 374 bp fragment of exon 7 using primers and PCR conditions by Chessa *et al.* (2005) in order to discriminate A/A1, C/C1, E, and 0' alleles. The second protocol was used to discriminate allele C to C1 amplifying a 325 bp fragment of exon 9 using primers by Chessa *et al.* (2008) and PCR conditions by Chessa *et al.* (2005) with an annealing temperature of 56°C. Finally, the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) protocol proposed by Cosenza *et al.* (2005) was used to discriminate allele A to A1. All PCR products were checked by electrophoresis on 2% agarose gel stained with SYBR Safe (Invitrogen, Carlsbad, CA, USA).

### Sequencing protocol

The PCR products were purified using PCR Product Clean-Up protocol as suggested by Fermentas (Hanover, MD, USA) using 10 U of

Exonuclease I and 1 U of FastAP Thermosensitive Alkaline Phosphatase (Fermentas). The resulting purified PCR products did not need additional purification before sequencing. Sequencing reactions were performed with BigDye v3.1 Cycle Sequencing Kit in an ABI PRISM 3130xl Genetic Analyser (Applied Biosystems, Carlsbad, CA, USA).

### Analysis of the sequences

SeqScape v3.1 software (Applied Biosystems) was used to analyse the nucleotide sequences. Alignments of the sequences were performed using ClustalW software (Thompson *et al.*, 1997). Polymorphic sites were confirmed by visual examination of the electropherograms. Allele and genotype frequencies and deviation from Hardy-Weinberg equilibrium were estimated using GENEPOP software version 4.0.11 (Rousset, 2008). Moreover, expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosity were calculated with POPGENE software version 1.31 (Yeh *et al.*, 1999).

## Results and discussion

Sequencing analysis and alignment of the obtained sequences showed the presence of A/A1, C, C1, and 0' alleles (Table 1) in *Girgentana* goat breed. All the individuals carrying A/A1 allele (n=9) were genotyped with PCR-RFLP protocol (Cosenza *et al.*, 2005), but the A1 allele was not found in our samples. Among the analysed samples and within the sequenced regions, no new polymorphisms were detected. The most frequent allele was C (0.597) followed by C1 (0.326), 0' (0.054), and A (0.023) (Table 2). These results are in agreement with those reported by Chessa *et al.* (2005) and Gigli *et al.* (2008) who described the predominance of C allele (together with C1 allele) in Italian and Sicilian goat breeds, respectively. Similar results were reported in Czech (Sztankóová *et al.*, 2008), Turkish and Indian goat breeds (Chessa *et al.*, 2007) for the C allele frequency. In a previous study, Marletta *et al.* (2005) reported only A\* (A+B+C) and 0' alleles in *Girgentana* goat breed. In contrast to Italian goat breeds, the A

allele was the most frequent in West African (Caroli *et al.*, 2007) and some Turkish, Indian and Sudanese (Chessa *et al.*, 2007) goat breeds.

The results of our study showed the presence of the silent allele C1 with high frequency (0.326) in *Girgentana* goat breed. Some differences could be highlighted between our data and those reported by Gigli *et al.* (2008) for this goat breed, and in particular for A and 0' allele frequencies that showed lower frequencies in our samples. Considering that the number of flocks and their geographical distribution have changed during these last years, the individual samples are not the same of previous works (Marletta *et al.*, 2005; Gigli *et al.*, 2008). These differences could be probably due to different number of analysed samples belonging to different herds.

Table 2 shows the seven genotypic classes found in *Girgentana* goat breed. The most frequent genotype was CC1 (0.423), followed by CC (0.326), C1C1 (0.110), and C0' (0.096). No AA nor 0'0' homozygous individuals were found. The average values of  $H_o$  and  $H_e$ , and P value associated with the null hypothesis of Hardy-Weinberg equilibrium (HWE) were estimated. The *Girgentana* goat breed is not in HWE ( $P < 0.05$ ) at this locus and this could be probably due to relatively high heterozygosity ( $H_e = 0.4337$  vs  $H_o = 0.5663$ ), or to high level of inbreeding ( $F_{is} = 0.140$ ) due to the presence of local bottlenecks (Mastrangelo *et al.*, 2013).

**Table 1. Alleles characterised at DNA level in goat CSN2 gene.**

Item	Gene and protein substitutions					Reference
	8561	8913	8915	8946	10562	
Nucleotide position	8561	8913	8915	8946	10562	-
Amino acid position	58	166	167	177	-	-
Exon (codon within exon)	7 (16)	7 (124)	7 (125)	7 (135)	9 (60)	-
Allele						
CSN2*A	A (Leu)	C (Ser)	C (Gln)	C (Ala)	C	Rando (1998)
CSN2*A1					T	Cosenza <i>et al.</i> (2005)
CSN2*C				T (Val)		Chessa <i>et al.</i> (2005)
CSN2*C1				T (Val)	T	Chessa <i>et al.</i> (2008)
CSN2*E	A	A (Tyr)				Caroli <i>et al.</i> (2006)
CSN2*0'			T (Stop)			Rando <i>et al.</i> (1996)
CSN2*0	(Stop)					Persuy <i>et al.</i> (1999)

**Table 2. Genotype and allele frequencies at CSN2 locus in *Girgentana* goat breed.**

Genotype	Individuals, n	Frequency	Allele	Frequency
A0'	2	0.010	A	0.023
AC	4	0.020	C	0.597
C0'	19	0.096	C1	0.326
CC	64	0.326	0'	0.054
AC1	3	0.015		
CC1	83	0.423		
C1C1	21	0.110		

## Conclusions

Our study showed the predominance of C and C1 strong alleles with high frequency (0.926) in *Girgentana* goat breed as previously reported in other studies (Chessa *et al.*, 2005; Gigli *et al.*, 2008); hence, our results could be considered as an upgrade of previous results. The presence of strong alleles at CSN2 gene in *Girgentana* goat breed could be useful for the production of milk with high protein content and good cheese-making properties (Ramunno *et al.*, 2007). Food business operators should consider the possibility of reviving interest in *Girgentana* goat milk using weak and null genotypes at CSN2 locus to make peculiar food products, such as drinking milk. Furthermore, considering that CSN2 locus is closely linked to CSN1S1, CSN1S2, and CSN3 loci, further studies are needed to determine the relationship among alleles at CSN2 locus and at the three other casein loci in order to include haplotype information in breeding programmes for conservation of *Girgentana* goat breed.

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