SHORT COMMUNICATION

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

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Abstract

Among calcium sensitive caseins, β-casein is the most abundant in goat milk, representing up to 50% of total casein content. The goat *β-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 *β-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 *β-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 β-lactoglobulin and α-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 *αs1-casein* (*CNS1S1*), *β-casein* (*CNS2*), *αs2-casein* (*CNS1S2*) and *κ-caseins* (*CSN3*) 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 β-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 β-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 *β-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 quantified, using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), then they were diluted to a final concentration of 50 ng/μL 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

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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 3130*xl* 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 (He) and observed (Ho) 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 0α 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 Ho and He, and P value associated with the null hypotesis 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 (He=0.4337 *vs* Ho=0.5663), or to high level of inbreeding (F_{is}=0.140) due to the presence of local bottlenecks (Mastrangelo *et al*., 2013).

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.

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

	Gene and protein substitutions					Reference
Item						
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 *et al.* (2005)
CSN2*C				T (Val)		Chessa *et al.* (2005)
CSN2*C1				T (Val)	T	Chessa *et al.* (2008)
CSN2*E	A	A (Tyr)				Caroli *et al.* (2006)
CSN2*0'			T (Stop)			Rando *et al.* (1996)
CSN2*0	(Stop)					Persuy *et al.* (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		

References

ASSONAPA, 2013. Available from: http://www.assonapa.it/norme_ecc/Consistenze_Caprini.htm

Caroli, A., Chiatti, F., Chessa, S., Rigagnese, D., Bolla, P., Pagnacco, G., 2006. Focusing on the goat casein gene complex. J. Dairy Sci. 89:3178-3187.

Caroli, A., Chiatti, F., Chessa, S., Rigagnese, D., Ibeagha-Awemu, E.M., Erhardt, G., 2007. Characterization of the casein gene complex in West African goats and description of a new α_{s1}-casein polymorphism. J. Dairy Sci. 90:2989-2996.

Chessa, S., Budelli, E., Chiatti, A.M., Cito, P., Bolla, P., Caroli A., 2005. Predominance of β-casein (CSN2) C allele in goat breeds reared in Italy. J. Dairy Sci. 88:1878-1881.

Chessa, S., Chiatti, F., Rigagnese, D., Ibeagha-Awemu, E.M., Ozbeyaz, C., Hassan, Y.A., Baig, M.M., Erhardt, G., Caroli, A., 2007. The casein genes in goat breeds from different continents: analysis by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). Ital. J. Anim. Sci. 6(Suppl.1):73-75.

Chessa, S., Rigagnese, F., Küpper, J., Pagnacco, G., Erhart, G., Caroli, A., 2008. Short communication: the β-casein (CSN2) silent allele C1 is highly spread in goat breeds. J. Dairy Sci. 91:4433-4436.

Chianese, L., Caira, S., Garro, G., Quarto, M., Mauriello, R., Addeo, F., 2007. Occurrence of genetic polymorphism at goat β-CN locus. p 69 in Proc. 5th Int. Symp. Challenge to Sheep and Goats Milk Sectors, Alghero, Italy.

Cosenza, G., Paciullo, A., Gallo D., Di Berardinno, D., Ramunno, L., 2005. A SspI PCR-RFLP detecting a silent allele at the goat CSN2 locus. J. Dairy Res. 72:456-459.

Ferretti, L., Leone, P., Sgaramella, V., 1990. Long range restriction analysis of the bovine casein genes. Nucleic Acids Res. 23:6829-6833.

Galliano, F., Saletti, R., Cunolo, V., Foti, S., Marletta, D., Bordonaro, S., D'Urso, G., 2004. Identification and characterization of a new β-casein variant in goat milk by high-performance liquid chromatography with electrospray ionization mass spectrometry and mass-assisted laser desorption/ionization mass spectrometry. Rapid Commun. Mass Sp. 18:1972-1982.

Gigli, I., Maizon, D.O., Riggio, V., Sardina, M.T., Portolano, B., 2008. Casein haplotype variability in Sicilian dairy goat breeds. J. Dairy Sci. 91:3687-3692.

Grosclaude, F., Joudrier, P., Mahé, M.F., 1978. Polymorphisme de la caséine αs2 bovine: étroite liaison du locus αs2-Cn avec les loci αs1-Cn, β-Cn et κ-Cn, mise en evidence d'une délétion dans le variant αs2-Cn. Ann. Génét. Sél. Anim. 10:313-327.

Hayes, H., Petit, E., Bouniol, C., Popescu, P., 1993. Localisation of the alpha-S2-casein gene (CASAS2) to the homologous cattle, sheep and goat chromosome 4 by in situ hybridization. Cytogenet. Cell Genet. 64:282-285.

Mahé, M.F., Grosclaude, F., 1993. Polymorphism of β-casein in Creole goat of Guadaloupe, evidence for a null allele. Genet. Sel. Evol. 25:403-408.

Marletta, D., Bordonaro, S., Guastella, A.M., Criscione, A., D'Urso, G., 2005. Genetic polymorphism of the calcium sensitive caseins in sicilian Girgentana and Argentata dell'Etna goat breeds. Small Ruminant Res. 57:133-139.

Mastrangelo, S., Tolone, M., Sardina, M.T., Di Gerlando, R., Portolano, B., 2013. Genetic characterization of the Mascaruna goat, a Sicilian autochthonous population, using molecular markers. Afr. J. Biotechnol. 12:3758-3767.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16:1215 (abstr.).

Neveu, C., Mollé, D., Moreno, J., Martin, P., Léonil, J., 2002. Heterogeneity of caprine beta-casein elucidated by RP-HPLC7MS: genetic variants and phosphorylations. J. Protein Chem. 21:557-567.

Persuy, M.A., Printz, C., Medrano, J.F., Mercier, J.C., 1999. A single nucleotide delection resulting in a premature stop codon is associated with marked reduction of transcripts from a goat β-casein null allele. Anim. Genet. 30:444-451.

Popescu, C.P., Long, S., Riggs, P., Womack, J., Schmutz, S., Fries, R., Gallagher, D.S., 1996. Standardization of cattle karyotype nomenclature: report of the committee for the standardization of the cattle karyotype. Cytogenet. Cell Genet. 74:259-261.

Ramunno, L., Mariani, P., Pappalardo, M., Rando, A., Capuano, M., Di Gregorio, P., Cosenza, G., 1995. Un gene ad effetto maggiore sul contenuto di caseina β nel latte di capra. pp 186-186 in Proc. 11th ASPA Nat. Congress, Grado, Italy.

Ramunno, L., Pauciullo, A., Mancusi, A., Cosenza, G., Mariani, P., Malacarne, M., 2007. Influence of genetic polymorphism of calcium-sensitive caseins on structural, nutritional, renneting and hypoallergenic properties of goat milk. Sci. Tecn. Latt. Cas. 58:257-271.

Rando, A., 1998. GenBank Accession no. AJ011018. Capra hircus csn2 gene, exons 1 to 9, allele A. Available from: http://www.ncbi.nlm.nih.gov/nuccore/AJ011018

Rando, A., Pappalardo, M., Capuano, M., Di Gregorio, P., Ramunno, L., 1996. Two mutations might be for the absence of β-casein in goat milk. Anim. Genet. 27:31.

Rijnkels, M., 2002. Multispecies comparison of the casein gene loci and evolution of casein gene family. J. Mammary Gland Biol. 7:327-345.

Roberts, B., Di Tullio, P., Vitale, J., Herir, K., Gerdon, K., 1992. Cloning of the goat beta casein gene and expression in transgenic mice. Gene 121:255-262.

Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Res. 8:103-106.

Sztankóová, Z., Kysel'ova, J., Kott, T., Kottova, E., 2008. Technical note: detection of the C allele of β-casein (CSN2) in Czech dairy goat breeds using LightCycler analysis. J. Dairy Sci. 91:1-5.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.D., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876-4882.

Yeh, F.C., Yang, R.C., Boyle, T., 1999. POPGENE 32. Version 1.31. Population genetics software. Available from: http://www.ualberta.ca/~fyeh/fyeh/