# Genetic polymorphisms of $\alpha_{s1}$-, $\alpha_{s2}$- and k-casein in Maltese goat breed

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***RIASSUNTO –*** Polimorfismo genetico di $\alpha_{S1}$-, $\alpha_{S2}$- e k-caseina nella capra Maltese – *È stata condotta un'indagine sulla capra Maltese per la tipizzazione genetica delle caseine. Sono stati analizzati mediante isoelettrofocalizzazione (IEF) 105 campioni individuali di latte. Su DNA estratto dal latte sono state eseguite ulteriori analisi. Al* locus *$\alpha_{S1}$-caseina sono stati osservati gli alleli A (frequenza = 0,381), B (0,148), E (0,057) ed F (0,414). Maggiormente polimorfico è risultato il* locus *$\alpha_{S2}$-caseina: A (0,548), B (0,062), C (0,319), G (0,067) e 0 (0,005). La k-caseina ha evidenziato, mediante analisi PCR-SSCP, la frequenza predominate (0,705) di un allele, chiamato D, che in IEF si sovrappone alla variante A. Sono stati identificati allo stesso* locus *gli alleli A (0,089) e B (0,203).*

**KEY WORDS:** goat, casein, genetic polymorphisms.

**INTRODUCTION** – The casein genes are organized as a tight cluster including *$\alpha_{S1}$- (CSN1S1), $\beta$- (CSN2), $\alpha_{S2}$- (CSN1S2)* and k-casein *(CSN3).* Investigation at casein haplotype level is therefore necessary to detect effects which could be used for the genetic improvement of goat breeds aiming to preserve biodiversity, safeguard typical products, and valorise nutritional properties of goat milk.

Mutations responsible for the reduced or null expression of *CSN1S1* (Leroux *et al.*, 1992; Jansa Perez *et al.*, 1994; Martin *et al.*, 1999), *CSN2* (Ramunno *et al.*, 1995) and CSN1S2 genes (Ramunno *et al.*, 2001) have been identified in goat. Moreover, recent studies on goat *CSN3* (Caroli *et al.*, 2001; Yahyaoui *et al.*, 2001; Angiolillo *et al.*, 2002; Jann *et al.*, 2003) showed that *CSN3* gene is highly polymorphic. The aim of this work was to describe the genetic variability of casein genes in the Southern Italy goat breed Maltese, in order to obtain all the information necessary for the definition of casein haplotypes.

**MATERIAL AND METHODS –** Individual milk samples were collected from a total of 105 Maltese goats reared in two flocks of Matera and Lecce provinces. Maltese breed is an Italian dairy goat, coming from the Middle East and spread first in Sicily (http://dad.fao.org). A screening of casein variability was performed at protein level by the isoelectrofocusing analysis (IEF) of the skimmed milk samples (Caroli *et al.*, 2001). Further typing was carried out at molecular level on the DNA extracted from milk, in order to detect alleles not identifiable by IEF (Table 1). Data were statistically analysed by GENEPOP program (Raymond and Rousset, 1995) to test Hardy-Weinberg and genotypic linkage *equilibrium*.

**RESULTS AND CONCLUSIONS** – The allelic frequencies at the typed loci are shown in Table 2. Hardy-Weinberg equilibrium was demonstrated for each *locus*, while highly significant linkage *disequilibrium* was found among the three casein *loci*.

The predominant allele at *CSN1S1* was the *F* allele, associated to a low content of $\alpha_{S1}$-casein, with a frequency slightly higher than the strong allele *CSN1S1*A* (0.414 *vs* 0.381). The *B* and *E* allele were also found with frequencies of 15% and 5.7% respectively, while no evidence of *CSN1S1*C* and *0* alleles was highlighted.

Table 1. Used techniques and detectable alleles.

| Technique | Level | Reference | *Locus* | Detectable alleles |
|---|---|---|---|---|
| IEF | Protein | Caroli *et al.*, 2001 | *CSN1S1* | *A, C, B/E* |
| | | | *CSN1S2* | *A, B, C, G* |
| | | | *CSN3* | *A/D*, B* |
| PCR-RFLP | DNA | Ramunno *et al.*, 2000 | *CSN1S1* | *F, A/0, B/E* |
| AS-PCR | DNA | Jansa Perez *et al.*, 1994 | *CSN1S1* | *E,* non *E* |
| AS-PCR | DNA | Cosenza *et al.*, 2001 | *CSN1S1* | *0,* non *0* |
| PCR-RFLP | DNA | Ramunno *et al.*, 2001 | *CSN1S2* | *A, 0* |
| PCR-SSCP | DNA | Caroli *et al.*, 2001 | *CSN3* | *A, B, D** |

** $CSN3^{D}$ variant is a further allele (Jann* et al.*, 2003) which is now identifiable by the SSCP analysis (see text).*

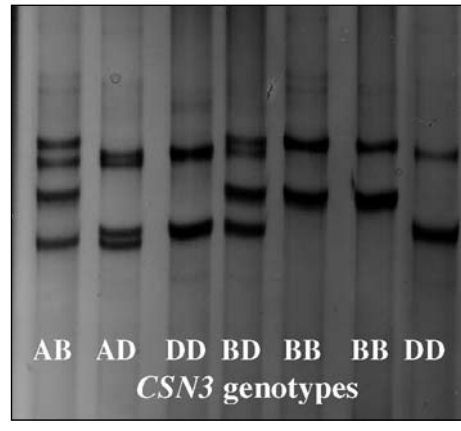
Table 2. Allelic frequencies at casein *loci*.

| CSN1S1 allele | Frequency | CSN1S2 allele | Frequency | CSN3 allele | Frequency |
|---|---|---|---|---|---|
| *A* | 0.381 | *A* | 0.548 | *A* | 0.089 |
| *B* | 0.148 | *B* | 0.062 | *B* | 0.203 |
| *E* | 0.057 | *C* | 0.319 | *D* | 0.708 |
| *F* | 0.414 | *G* | 0.067 | | |
| | | 0 | 0.005 | | |

At *CSN1S2 locus,* the most common allele was *A* (55%), followed by *C* (32%). The *G, B* and *0* variants were also found, but with lower frequencies.

A very high frequency was shown for the *CSN3*D* allele (71%). This variant cannot be distinguished from *CSN3*A* at protein level. It was recently sequenced (GenBank accession no AY166705) and named *CSN3*D* in a phylogenetic study on the domestic and wild goat *CSN3* variability (Jann *et al.*, 2003) which made also an effort to standardise the conflicting nomenclature actually existing on caprine *CSN3* genetic polymorphisms. By PCR-SSCP analysis (Figure 1), *CSN3*D* shows a migration pattern quite dif-

Figure 1. SSCP analysis of *CSN3 locus* allowing the separation of *A, B*, and *D* alleles.



ferent from the A variant, corresponding to the goat *CSN3* sequence of Coll *et al.* (1993) (GenBank accession n° X60763). The "true" *CSN3*A* allele was also found in Maltese breed, but at a rather low frequency (8.9%). A higher frequency was detected for *CSN*B* (20%). Moreover, two further SSCP patterns were found in three animals typed for *CSN3* and showing an *IEF* migration corresponding to the *A*/*D* variants. The new DNA patterns are under further molecular characterisation.

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**REFERENCES** – **Angiolillo**, A., Yahyaoui, M.H., Sanchez, A., Pilla, F., Folch, J.M., 2002. J. Dairy Sci. 85:2679–2680. **Caroli**, A., Jann, O., Budelli, E., Bolla, P., Jäger, S., Erhardt, G., 2001. Anim. Genet. 32:226-230. COLL, A., Folch, J.M., Sanchez, A., 1993. J. Anim. Sci. 71:2833. **Cosenza**, G., Pappalardo, M., Pastore, N., Rando, A., Di Gregorio, P., Masina, P., Ramunno, L., 2001. pp. 65-66 in Proc. XIV Nat. Cogr. A.S.P.A., Firenze, Italy. **Jann**, O., Prinzenberg, E.M., Luikart, G., Caroli, A., Erhardt, G., 2003. Submitted. **Jansa Perez**, M., Leroux, C., Sanchez Bonastre, A., Martin, P., 1994. Gene 147:179-187. **Leroux**, C., Mazure, N., Martin, P., 1992. J. Biol. Chem. 267:6147-6157. **Martin**, P., Ollivier-Bousquet, M., Grosclaude, F., 1999. Inter. Dairy J. 9:163-171. **Ramunno**, L., Cosenza, G., Pappalardo, M., Pastore, N., Gallo, D., Di Gregorio, P., Masina, P., 2000. Anim. Genet. 31:342-343. **Ramunno**, L., Longobardi, E., Pappalardo, M., Rando, A., Di Gregorio, P., Cosenza, G., Mariani, P., Pastore, N., Masina, P., 2001. Anim. Genet. 32:19-26. **Ramunno**, L., Mariani, P., Pappalardo, M, Rando, A., Capuano, M., Di Gregorio, P., Cosenza, G., 1995. pp. 185-186 in Proc. XI Nat. Congr. A.S.P.A., Grado (GO), Italy. **Raymond**, M., Rousset, F., 1995. J. Hered. 86:248-249. Yahyaoui, M.H., Coll, A., Sanchez, A., Folch, J.M., 2001. J. Dairy Res. 68:209-216.