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# Study on genetic variability at $\alpha_{s1}$ and $\alpha_{s2}$ casein loci in autochthonous goat populations of the Lombardy Alps

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***RIASSUNTO*** – Studio sulla variabilità genetica ai loci $\alpha_{S1}$ e $\alpha_{S2}$ caseina in popolazioni autoctone delle Alpi lombarde – *E' stato studiato il polimorfismo ai* loci *$\alpha_{S1}$e $\alpha_{S2}$ caseina, tramite PCR, su DNA genomico di 284 capre di razza Bionda dell'Adamello e Lariana. Sono state osservate 26 combinazioni genotipiche, tra le quali la più frequente è* CSN1S1$^{FF}$/CSN1S2$^{AA}$ *(44,4%), i cui alleli ai due* loci *sono i più diffusi (69,7 e 88,2%). Tutte le altre combinazioni sono molto meno frequenti, a partire da* CSN1S1$^{EF}$/CSN1S2$^{AA}$ *con il 12,7%. In 237 soggetti con aplotipi informativi (omozigoti ad almeno uno dei due* loci*), si è rilevato che gli aplotipi* CSN1S1$^{F}$/ CSN1S2$^{B}$ e CSN1S1$^{0}$/CSN1S2$^{A}$ *sono meno frequenti dell'atteso mentre l'aplotipo* CSN1S1$^{0}$/ CSN1S2$^{B}$ *è più frequente dell'atteso (P<0,05).*

**KEY WORDS:** autochthonous goats, casein polymorphism, haplotype

**INTRODUCTION** – In cattle, sheep and goats (Hayes *et al.*, 1993) the casein genes form a cluster, on the chromosome 6, of four tightly linked genes, whose sequence is: $\alpha_{S1}$-β-$\alpha_{S2}$-k (Threadgill and Womack, 1990; Ferretti *et al.*, 1990; Gallagher *et al.*, 1994; Rijnkles *et al.*, 1997). For this reason the alleles at these *loci* are generally inherited all together (Di Stasio and Mariani, 2000), with low recombination frequency. Thus it is worth noting the importance of describing the casein haplotypes namely the inherited combination of closely linked alleles (King and Stansfield, 1985; Meuwissen and Goddard, 2001). The knowledge of the casein allele associations could be important for the selection. In goat milk the casein alleles are related to important differences in the level of synthesis of the respective gene products and in milk chemical-physical properties (Rando *et al.*, 2000). The $\alpha_{S1}$-casein alleles are related to 4 synthesis levels (strong: A, B and C alleles, 3.4 g/l; intermediate: E allele, 1.1 g/l; weak: D, F and G alleles, 0.45 g/l; null: 0 allele), the β-casein alleles to 2 synthesis levels (5.0 and 0 g/l for the A, B and null allele respectively) and the $\alpha_{S2}$-casein alleles to 2 levels (2.0 and 0 g/l for the alleles A, B, C, E, F and 0 respectively). Thus the possible combinations between the different synthesis levels at the three loci are 16, but the haplotypes that are observed in a population are generally much less, due to *linkage disequilibrium* and to the very low frequency of some alleles.

Aim of this work was the study of the association between alleles at the *loci* CSN1S1 and CSN1S2 in goats from two autochthonous populations of the Lombardy Alps: the Lariana (or Valle di Livo goat) and Bionda of Adamello.

**MATERIAL AND METHODS** – Blood samples were taken from 138 Bionda of Adamello goats in 21 flocks and from 146 Lariana goats in 9 flocks. Genomic DNA was extracted using the Nucleospin kit *(Macherey-Nagel)*. Five different PCR protocols were performed to detect CSN1S1 and CSN1S2 polymorfisms by means of PCR-RFLP for CSN1S1$^{F}$ (Ramunno *et al.*, 2000), PCR for CSN1S1$^{E}$ (Jansa Perez *et al.*, 1994), AS-PCR for CSN1S1$^{0}$ (Cosenza *et al.*, 2001), ASM-PCR for CSN1S2$^{B,C}$ and PCR-RFLP for CSN1S2$^{0}$ (Ramunno *et al.*, 2001a,b). No distinction was made among the three strong CSN1S1 alleles (A, B and C) that were assigned by exclusion, after the already described PCR. Also the CSN1S2$^{A}$ allele was assigned by exclusion. The very rare alleles CSN1S1$^{D,G}$ and CSN1S2$^{E,F}$ were not considered.

**RESULTS AND CONCLUSIONS** – Among the genotypes at CSN1S1 and CSN1S2 *loci*, shown in Table 1, only 26 composite genotypes have been observed. The most frequent combination is $CSN1S1^{FF}/CSN1S2^{AA}$ (44.4%) homozygous at both *loci*. The involved alleles, $CSN1S1^{F}$ and $CSN1S2^{A}$, are the most frequent in the whole sample (69.7 and 81.7% respectively). Other less frequent combinations are: $CSN1S1^{EF}/CSN1S2^{AA}$ (12.7%), $CSN1S1^{F0}/CSN1S2^{AB}$ (10.9%), $CSN1S1^{Fstrong}/CSN1S2^{AA}$ (6.7%), $CSN1S1^{FF}/CSN1S2^{AB}$ (3.9%) and $CSN1S1^{00}$/ $CSN1S2^{BB}$ (3.5%). All the other combinations have very low frequencies. The associations between alleles at the two *loci* was estimated in a subset of subjects (no.=237) that, being homozygous for at least one *locus*, have informative haplotypes (in italics in tab. 1). The allelic frequencies at the two *loci* in this data subset were used to estimate the expected frequencies of each haplotype in the hypothesis of *linkage equilibrium* of the two *loci* (Tab. 2).

Table 1. Observed genotypes and genotype frequencies at CSN1S1-CSN1S2 *loci*.

| CSN1S2<br>CSN1S1 | AA<br>no. | AO<br>no. | AB<br>no. | AC<br>no. | BB<br>no. | BC<br>no. | Total | CSN1S1<br>(%) |
|---|---|---|---|---|---|---|---|---|
| F strong | *19* | | 1 | 2 | | | 22 | 7.75 |
| EE | *4* | | *7* | *1* | | | 12 | 4.22 |
| E0 | | | 4 | 1 | | | 5 | 1.76 |
| EF | *36* | | 2 | 3 | | | 41 | 14.44 |
| FF | *126* | *1* | *11* | *5* | *1* | | 144 | 50.70 |
| F0 | *8* | | 31 | 1 | *4* | 1 | 45 | 15.85 |
| 00 | *1* | | 1 | | *10* | | 12 | 4.22 |
| 0 strong | *2* | | 1 | | | | 3 | 1.06 |
| Total | 196 | 1 | 58 | 13 | 15 | 1 | 284 | |
| CSN1S2 (%) | 69.02 | 0.35 | 20.49 | 4.54 | 5.28 | 0.35 | | 100 |

In the above mentioned subset only 10 of the 16 possible haplotypes were observed. Four of the 6 absent haplotypes ($CSN1S1^{strong}/CSN1S2^{C}$; $CSN1S1^{strong}/CSN1S2^{0}$; $CSN1S1^{0}/CSN1S2^{C}$ and $CSN1S1^{0}/CSN1S2^{0}$) were neither observed in the whole 284 animal set. This is related to the low allelic frequency of the $CSN1S1^{strong}$ (4.4%), $CSN1S2^{C}$ (2.5%) and $CSN1S2^{0}$ (0.2%) alleles in these goats. The difference between the observed and the expected haplotype frequencies at the $\chi^2$ test were significant ($P<0.001$) and in agreement with the *linkage* existing between the two considered *loci*. In particular, the haplotypes $CSN1S1^{F}/CSN1S2^{B}$ and $CSN1S1^{0}/CSN1S2^{A}$ were less frequent than expected, while there was an excess of $CSN1S1^{0}/CSN1S2^{B}$ ($P<0.01$).

No one of these haplotypes should change significantly the global percentage of the respective proteins in the milk. In particular, according to the value reported by Rando *et al.* (2000) and considering that in these goats the β-casein allele 0 is absent (data not shown, found by milk IEF), the level of Ca-sensitive caseins induced by the favoured haplotype $CSN1S1^{0}/CSN1S2^{B}$ should be 7 g/l, including the β-casein. This level is similar to or slightly lower than the one of the haplotypes resulted to be less frequent than expected.

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Table 2. Observed and expected frequencies of informative haplotypes.

| Allele frequencies (%) | | Haplotype CSNS1/S2 | Obs. (no.) | Exp. (no.) | Haplotype CSNS1/S2 | Obs. (no.) | Exp. (no.) |
|---|---|---|---|---|---|---|---|
| $CSN1S1^{strong}$ | 4.4 | Strong/A | 21 | 18.5 | E/A | 52 | 52.9 |
| $CSN1S1^{F}$ | 74.9 | Strong/B | 0 | 2.2 | E/B | 7 | 6.2 |
| $CSN1S1^{E}$ | 12.7 | Strong/C | 0 | 0.3 | E/C | 1 | 0.8 |
| $CSN1S1^{0}$ | 8.0 | Strong/0 | 0 | 0.1 | E/0 | 0 | 0.1 |
| $CSN1S2^{A}$ | 88.2 | F/A | 332 | 313.1 | **0/A** | **13** | **33.5** |
| $CSN1S2^{B}$ | 10.3 | **F/B** | **17** | **36.7** | **0/B** | **25** | **3.9** |
| $CSN1S2^{C}$ | 1.3 | F/C | 5 | 4.5 | 0/C | 0 | 0.1 |
| $CSN1S2^{0}$ | 0.2 | F/0 | 1 | 1.2 | 0/0 | 0 | 0.1 |

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