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Genetic variability of the ovine α_{s1}-casein

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RIASSUNTO – Variabilità genetica dell'αs1-caseina ovina. *La variabilità genetica dell'α_{s1}-caseina ovina è stata valuta con particolare attenzione alla presenza delle varianti A, C, e D e del polimorfismo nell'esone 17 che determina una transizione T→C a cui corrisponde la sostituzione aminoacidica Ile_{186}→Thr_{186}. La variante C è stata suddivisa in due tipi, C' e C", in base all'aminoacido presente in posizione 186. L'aplotipo ancestrale è C' (Pro_{13}-Ser_{68}-Thr_{186}) mentre C" (Pro_{13}-Ser_{68}-Ile_{186}) è risultato il più frequente in 5 razze ovine italiane.*

KEY WORDS: α_{s1}-casein, variability, intragenic haplotype, ovine.

INTRODUCTION – The casein genetic polymorphisms are important for their effects on quantitative traits and technological properties of milk. At the α_{s1}-casein (*CSN1S1*) level three genetic variants were characterised (*A, C, D*) in ovine milk (Ferranti *et al.*, 1995). The *C* variant differs from *A* by having a Pro instead of Ser at position 13, whereas the *D* variant is characterized by a further substitution at position 68 (Ser_{68}→Asn_{68}). A negative effect of *D* variant on milk technological and compositional properties was also described (Piredda *et al.*, 1993, Pirisi *et al.*, 1999). Recently (Ceriotti *et al.*, 2004b), a transition T→C has been found in exon 17, resulting in the amino acid exchange Ile_{186}→Thr_{186}, not identifiable at the protein level by standard typing methods, but detectable at the DNA level by Polymerase Chain Reaction – Single Strand Conformation Polymorphism (PCR-SSCP).
The aim of this work was to study the organisation and variability of the intragenic haplotypes responsible for the three amino acid substitutions in order to suggest a phylogenetic hypothesis. The investigation involved five Italian ovine breeds. Three of them were preliminarily described (Ceriotti *et al.*, 2004a), while two others were included in the present study.

MATERIAL AND METHODS – A total of 190 milk and blood samples were analysed from Comisana (CO; n=66), Gentile di Puglia (GP; n=20), Massese (MA; n=24), Sarda (SA; n=45), and Sopravissana (SO; n=35) breeds. DNA was extracted from blood with a commercial kit (GFX™ Genomic Blood DNA Purification Kit, Amersham Biosciences, Piscataway N.J). Milk was analysed by isoelectric focusing (IEF; Chessa *et al.*, 2003) in order to identify the genetic variants *A*, *C*, and *D*. At the DNA level a fragment of around 800 bp, containing part of exon 9, intron 9 and part of exon 10, was examined by PCR-Allele Specific (Ramunno *et al.*, 1997), while a fragment of 223 bp in exon 17 was analysed by PCR-SSCP (Ceriotti *et. al.*, 2004b). Moreover, exon 3 polymorphism was analysed at the DNA level (Pilla *et al.*, 1998). Gene frequencies and genotypic combinations were evaluated by direct count. Informative haplotypes were used to calculate the number of haplotypes surely observed in each breed.

RESULTS AND CONCLUSIONS – Full agreement was found between IEF analysis and typing performed at the DNA level for the identification of *A*, *C*, and *D* variants. No ewe carried the *CSN1S1*A* allele,

so the analysis of the intragenic haplotype structure was performed for the genetic polymorphism at exon 9, differentiating between *D* and *non D*, and exon 17, identifying the presence of the nucleotide T or C in the triplet coding for amino acid at 186 position.

Table 1 shows the absolute frequencies of different genotypic combinations in the five breeds. No subject was found presenting the combination *CD*-CC. The *D* variant did not occur in the Sarda sample, and was found at a rather low frequency in the other breeds, in ascending order: 0.02 (CO), 0.04 (MA), 0.05 (GP), 0.09 (SV). As for the exon 17 polymorphism, T nucleotide showed a higher frequency than C (GP and MA=0.65; CO=0.73; SV=0.81; SA=0.89).

Table 1. Genotypic combinations (absolute frequencies) for *CSN1S1* exon 9 and exon 17 polymorphisms.

exon 9	exon 17	CO	GP	MA	SA	SV	TOTAL
CC	TT	34	7	9	37	19	106
CC	CT	25	9	10	6	10	60
CC	CC	5	2	3	2	0	12
CD	TT	1	1	1	0	3	6
CD	CT	1	1	1	0	3	6
TOTAL		66	20	24	45	35	190

Table 2 shows the number of intragenic haplotypes for each breed, calculated on the basis of informative genotype combinations (all the ones reported in table 1 except *CD*-CT). The most common haplotype is *C*-T, followed by *C*-C. Only 6 chromosomes surely carried *D*-T haplotype, while *D*-C, not found in Sarda, was not evaluated in the other breeds since its occurrence could only be deduced from the non informative combination *CD*-CT.

Table 2. Number of haplotypes deduced from the informative genotype combinations.

HAPLOTYPE exon 9 - exon 17	CO	GP	MA	SA	SV	TOTAL
C-T	94	24	29	80	51	278
C-C	35	14	16	10	10	85
D-T	1	1	1	0	3	6
D-C	-	-	-	0	-	-
TOTAL	130	40	46	90	64	370

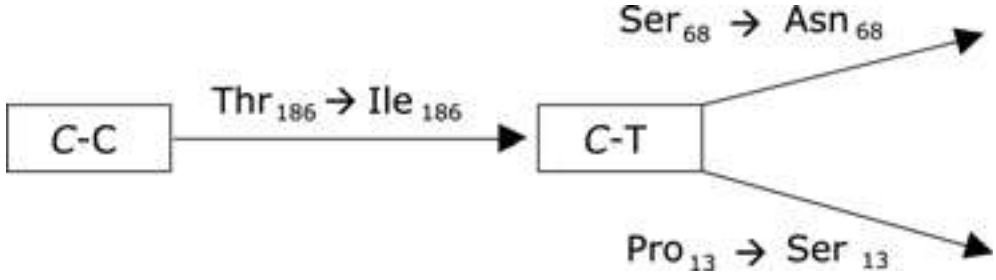
The alignment of protein sequences available on SwissProt, P04653 (ovine CSN1S1), P18626 (goat CSN1S1) and P02662 (bovine CSN1S1) indicates that the ancestral amino acid in the ovine protein are Pro_{13}, Ser_{68} e Thr_{186}. Out of the intragenic haplotype observed, the ancestral is *C*-C (Pro_{13}-Ser_{68}-Thr_{186}), from which *C*-T (Pro_{13}-Ser_{68}-Ile_{186}) and successively *D*-T (Pro_{13}-Asn^{68}-Ile_{186}) derived.

In addition, the primary protein sequences analysed by Ferranti *et al.* (1995) indicates the occurrence of Ile at position 186 in the variants *A*, *C* and *D*. Table 3 shows the haplotype combinations of the three variants, on the basis of both the present work and of the previous protein sequencing. While for *A* and *D* variants only one intragenic haplotype occurs (Ser_{13}-Ser_{68}-Ile_{186} and Pro_{13}-Asn_{68}-Ile_{186}), for the *C* variant two different intragenic haplotypes exist, which can be defined as *C'* and *C"* taking into account of the evolutive trend: Pro_{13}-Ser_{68}-Thr_{186} and Pro_{13}-Ser_{68}-Ile_{186}. The first haplotype, *C'*, is the ancestral one, according to the evolutive model proposed in figure 1 and supported by the present findings. The second haplotype, *C"*, is the most common in the analysed breeds. We can thus conclude that at least four protein variants occur at the ovine *CSN1S1* locus: *A*, *C'*, *C"*, and *D*.

Table 3. *CSN1S1* variants until now found, with the novel polymorphism at 186 position. The ancestral amino acid is bolded. (*): the amino acid was observed in the sequencing of the primary protein structure performed by Ferranti *et al.* (1995).

	Amino acid position		
CSN1S1 variant	13	68	186
A	Ser	**Ser**	Ile (*)
C	**Pro**	**Ser**	Ile / **Thr**
D	**Pro**	Asn	Ile

Figure 1. Possible phylogenetic pattern of *CSN1S1* intragenic haplotypes (exon 9 and 17).



Further studies should assess the distribution of CSN1S1 intragenic haplotypes in a wider sampling, comprehensive also of other ovine breeds. The eventual occurrence of micro-recombination and gene conversion phenomena should be elucidated. Moreover, the effects of the intragenic haplotypes on productive traits, mainly milk qualitative and technological characteristics, are to be investigated for possible useful implications in the genetic improvement of the ovine breeds.

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