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Polymorphism of Genetic variability of gene in sheep

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RIASSUNTO – Il polimorfismo del gene MUC1 nella pecora. *Il gene MUC1 esprime una mucina della membrana dei globuli di grasso del latte e contiene una regione ripetitiva ipervariabile. La sequenza di MUC1 è nota nel bovino e nella capra ma non nella pecora. Un'analisi preliminare della regione ripetitiva, eseguita mediante PCR su 23 soggetti di razza Tacola utilizzando una coppia di oligonucleotidi disegnati sulla sequenza di capra, ha evidenziato il polimorfismo di lunghezza già noto per MUC1 di uomo, bovino e capra. L'analisi della sequenza di un frammento ha dimostrato l'esistenza di una regione ripetitiva formata da un'unità di 60 bp ripetuta in successione, caratteristica in comune con il gene MUC1 delle altre specie. I risultati ottenuti sono il primo contributo alla conoscenza di MUC1 di pecora e consentono di avviare indagini sul suo polimorfismo nelle razze ovine.*

KEY WORDS – heep, MUC1, minisatellite, polymorphism.

INTRODUCTION – MUC1 gene encodes the polypeptide of a mucin present on the apical surface of the secreting mammary cells during lactation and on the surface of the milk fat globules (Mather, 2000). MUC1 contains a minisatellite region: the sequence, coding for the extracellular protein domain, consists mostly of a motif of 60 bp that in human, cattle and goat is tandemly repeated a variable number of times (VNTR polymorphism), giving rise to molecular variants of different size (Patton *et al.*, 1995).

The interest that MUC1 mucin holds in dairy species derives from possible relationship among the length of variants and important traits like the mechanism of lipid secretion, the milk fat content and the resistance to mastitis determinants (Hens *et al.*, 1995; Patton, 1999; Lillehoj *et al.*, 2001).

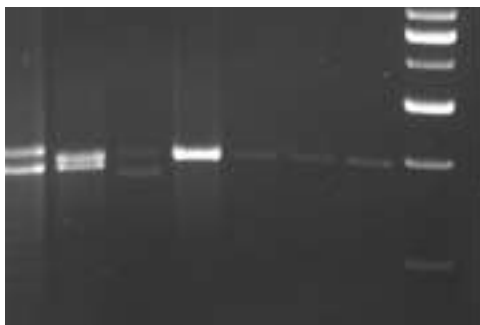
In previous investigations we analysed the structure of the MUC1 gene in cattle and goat, obtaining sequences of the repetitive region and of the flanking unique-sequence regions (Rasero *et al.*, 2002; Sacchi *et al.*, 2004). On the other hand, there is still a lack of information concerning the MUC1 gene and its polymorphism in sheep.

An investigation was undertaken with the aim of characterizing the repetitive region of MUC1 gene in sheep.

MATERIAL AND METHODS – Blood samples were collected from 23 Tacola sheep. Genomic DNA was obtained from 200 µl of whole blood using the Nucleospin Blood Pure method (Macherey-Nagel GmbH, Dueren, Germany). PCR reactions were carried out by a GeneAMP PCR System 2400 thermal *cycler* (Applied Biosystem, Foster City, CA), using two primers, PG4 and PG9, derived from the goat MUC1 sequences, upstream and downstream the repetitive region respectively (Sacchi *et al.*, 2004). The PCR fragments were resolved on agarose gel, purified and then cloned in the vector pDrive Cloning Vector (QIAGEN S.P.A., Milano, Italy). The cloned fragments were sequenced on an ABI PRISM 310 Genetic Analyzer (Applied Biosystem, Foster City, CA). Sequences were compared and aligned using the ClustalW program (Thompson *et al.*, 1994), and the primary structure of the corresponding polypeptide was deduced using the GENSCAN program (Burge and Karlin, 1997).

RESULTS AND CONCLUSIONS – Four different PCR fragments, with an average length equal to 1500 bp, were identified on agarose gel, each individual sheep showing one or two bands, in accordance with the polymorphic model previously observed for MUC1 gene of human, cattle and goat (figure 1).

Figure 1. Agarose gel resolution of PCR fragments obtained from Tacola sheep samples: ladder Gibco 1kb, from top to bottom 3.5, 3, 2.5, 2, 1.5, 1 kb.



The sequence of one fragment showed a high degree of homology with the MUC1 sequences of cattle (AF399757) and goat (AY388993 and AY388994). This fragment contains a repetitive region and two unique-sequence flanking regions. The 5' end of the fragment is 90% and 95% homologous with cattle and goat sequences respectively, while the 3' end shows homology equal to 91% and 99% with cattle and goat, respectively.

It was not possible to obtain completely overlapping sequences from the two most internal primers because of the length of the repetitive region. The analysis of the sequence indicated that the repetitive region of MUC1 gene in sheep is an array of 60 bp units tandemly repeated, as in human, cattle and goat. The average homology of the repeated units with the consensus sequence, obtained by their alignment, is equal to 87%.

In figure 2 the sheep consensus sequence is compared with those of goat (Sacchi *et al.*, 2004) and cattle (Rasero *et al.*, 2002). Sheep sequence differs from the sequences of goat and cattle by two and five nucleotides, respectively. Thus the homology degree between sheep and goat is 97% whereas between sheep and cattle is 91%.

Figure 2. Alignment of consensus repetitive units of sheep, goat and cattle (from top to bottom).

1	10	20	30	40	50	60
I	I	I	I	I	I	I
AGCCCGGCTGCCTCTCCAGGCCACGACGGTGCCTCGTCCCCAACCCAGCAGCCCTGCCCCG						
AGCCCGGCTGCCTCTCCAGGCCACGACGGCGCCTCGACCCCCAACCCAGCAGCCCTGCCCCG						
AGCCCGGCTGCCTCTCCAGGCCACGACGGAGCCTCGACTCCAACCCAGCAGCCCTGCCCCA						
*****	*****	*****	*****	*****	*****	*****

The alignment of the corresponding polypeptides shows that the polypeptide coded by the sheep consensus sequence is very alike with the homologous sequences of cattle and goat, as they share 95% of aminoacids. Only one of the observed nucleotide substitutions involves the presence of a different aminoacid residue (figure 3). The resulting substitution, ser/thr does not change the functional significance of the residue. In particular, seven residues of ser/thr, potential glycosylation sites, result highly conserved. The obtained results represent the first contribution to the knowledge of the structure of the sheep MUC1 gene and will allow to elaborate specific analytic protocol for investigating its polymorphism in different breeds.

Figure 3. Alignment of polypeptides coded by the consensus sequences.

<i>Ovis aries</i>	S	P	A	A	S	P	G	H	D	G	A	S	S	P	T	S	S	P	A	P
<i>Capra hircus</i>	S	P	A	A	S	P	G	H	D	G	A	S	T	P	T	S	S	P	A	P
<i>Bos taurus</i>	S	P	A	A	S	P	G	H	D	G	A	S	T	P	T	S	S	P	A	P

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