Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

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To cite this article: G. Cosenza, A. Pauciullo, R. Illario, D. Gallo, D. Di Berardino, D. Nicodemo & L. Ramunno (2005) A preliminary analysis of the goat lactoferrin encoding gene, Italian Journal of Animal Science, 4:sup2, 49-51, DOI: 10.4081/ijas.2005.2s.49

To link to this article: https://doi.org/10.4081/ijas.2005.2s.49

A preliminary analysis of the goat lactoferrin encoding gene

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RIASSUNTO – Analisi preliminare del gene che codifica la lattoferrina caprina. *È stato sequenziato il tratto di DNA comprendente gli ultimi 30 nucleotidi del 2° introne e i primi 52 nucleotidi del 6° introne (per un totale di 2824 bp) del gene della lattoferrina (Lf) di 3 capre appartenenti rispettivamente alla razza Saanen, Maltese e ad una popolazione autoctona allevata in provincia di Catanzaro (Italia). Il confronto tra le sequenze ottenute ha evidenziato 8 siti polimorfici (6 transversioni e 2 transizioni realizzatesi a livello intronico), mentre il confronto con le sequenze dei cDNA della Lf caprina depositate in Banca Dati mostra 5 sostituzioni nucleotidiche responsabili di tre sostituzioni aminoacidiche. In generale, il gene Lf caprino presenta una struttura simile a quella dell'omologo gene nella specie bovina, fatta eccezione per l'inserzione in quest'ultima specie di una sequenza di origine retroposonica (Bov A) a livello del 4° introne.*

KEY WORDS: *Capra hircus*, lactoferrin, milk protein, gene.

INTRODUCTION – Lactoferrin (*Lf*) is a glycoprotein with a molecular weight of ~80 kDa. It has been isolated from milk and identified in various different mammalian secretions. Several physiological functions have been ascribed to *Lf*, including regulation of cellular growth and differentiation (Iyer and Lonnerdal, 1993), intestinal iron homeostasis (Levay and Viljoen, 1995), host defense against microbial infection and inflammation (Baveye *et al.* 1999), regulation of myelopoiesis (Brock, 2002) and protection against cancer (Ward *et al.* 2002). The coding sequence of bovine lactoferrin gene is spread over 17 exons and 16 introns, spanning 34.5 kb of genomic DNA. The bovine and caprine lactoferrin gene was mapped to chromosome 22 (Schwerin *et al.*, 1994). To date, only the nucleotide sequence of the goat *Lf* cDNA, 2333 bp in length, is known which contains 75 bp of 5' UTR (Un Traslated Region), the ORF (Open Reading Frame) coding 690 aminoacid of mature protein and the whole 3' UTR (Le Provost *et al.*, 1994; Lee *et al.*, 1997). The leader peptide from 19 full-length is codified by the last 14 codons of the exon 1 and the first five of the exon 2. The aim of this study was to carry out a preliminary analysis of the goat lactoferrin encoding gene, focusing on the region spanning from the 3rd to the 6th exon.

MATERIAL AND METHODS – For this study three different genetic types of goats (Saanen, Maltese and an autocton population reared in province of Catanzaro, Italy) were used. Genomic DNA was isolated from leucocytes. DNA regions spanning from the 2nd exon to 7th exon of the goat *Lf* gene were amplified using a Gene Amp PCR System 2400 (Perkin Elmer). Primers for amplification and sequencing were designed by means of DNASIS-Pro software (Hitachi), using the Lf cDNA sequences (EMBL n° U53857): Lf2F (5'-GCCCCGAGGAAAAACGT-3'), Lf5R (5'-TTGAAGGCACCAGAATAAC-3'); Lf5F (5'-TGTGGCTAGATTCTTCTC-3'), Lf6R (5'-CAAACACTGTCGTCTCC-3'); Lf6F (5'-GGAGACGACAGTGTTTG-3'), Lf7R (5'-AACAGCATGAGAAGGGA-3'). A typical 50 µl of reaction mix comprised: 100 ng of genomic DNA, 3 mM $MgCl_2$, 200 nmol of each primer,

dNTPs each at 400 μM, Buffer 1X and 2.5 U of *Taq* DNA Polymerase (Promega), 0.04% BSA. The thermal profile consisting of a total of 31 cycles involving 1 cycle at 97°C for 2 min, 46-62°C for 45 sec and 72°C for 2 min followed by 30 cycles at 94°C for 45 sec, 46-62°C for 45 sec and 72°C for 2 min, ending with a final extention at 72°C for 10 min. PCR products were analysed by electrophoresis using a 1.5% agarose gels (Biorad). Nucleotide sequencing was carried out according to the dideoxynucleotide chain-termination technique

RESULTS AND CONCLUSION – We amplified by means of PCR and sequenced the DNA region spanning the last 30 nucleotides of the 2nd intron to the first 52 nt of the 6th intron (2824 bp) of the goat *Lf* encoding gene. A comparison of the same sequenced regions for the three samples has shown a similarity of 99.3%, with a 73.8% homology with the corresponding bovine sequence. At a preliminary analysis, the goat *Lf* encoding gene shares a similar organization with the known bovine counterpart (Seyfert *et al.*, 1994) and its architecture seems to be extremely split, according to the structure of the αs1 and αs2 caseins encoding genes (*CSN1S1* and *CSN1S2*, respectively) (Groenen *et al.*, 1993; Ramunno *et al.*, 2004). A comparison of the intronic sequenced regions has shown for the three goats the presence of 8 intronic polymorphic sites: 6 transversions and 2 transitions (Table 1).

Table 1. Intronic differences found in *Lf* gene of the three examined goats. Numbers are relative to nucleotide position in corresponding introns.

Intron	Position	Saanen	Maltese	Autocton
3	195	C	C	G
5	1181	C	C	G
	1197	C	C	G
	1198	C	C	G
	1257	G	C	G
	1420	C	C	T
	1424	C	T	C
	1611	C	A	C

The mutations are probably not responsible for any difference in *Lf* gene expression since they do not affect splicing sites, which follow the 5' GT/3' AG splice rule. At the 3rd intron, between nucleotides + 249 and + 260, a microsatellite sequence was evidenced; it is a $(CT)^6$ repetition that hasn't shown polymorphism in the three samples. Concerning the exonic regions, we sequenced the exons 3 (109 bp), 4 (183 bp), 5 (148 bp) and 6 (56 bp). A comparison of the sequenced exonic region with the published sequence of the French goat cDNA (Le Provost *et al.*, 1994) showed five nucleotide differences which result in three aminoacid substitutions. All the exonic differences are already known and it differenced the French from the Korean goat (Lee *et al.*, 1997) (Table 2).

Table 2. Exonic differences of the goat *Lf* gene evidenced by the comparison of the obtained sequences with those of the cDNA deposited in EMBL (n° X78902[a]; U53857[b]). Numbers are according to the published sequences.

Exon	Position	Italian goats		French goat[a]		Korean goat[b]	
		DNA	Protein	DNA	Protein	DNA	Protein
3	56	C**T**G	Leu	C**G**G	Arg	C**T**G	Leu
4	54	**C**AG	Gln	**A**AG	Lys	**C**AG	Gln
	74	GG**C**	Gln	GG**T**	Gln	GG**C**	Gln
	144-145	**TT**C	Phe	**CC**C	Pro	**TT**C	Phe

Finally the analysis of the partial goat *Lf* gene, from the 1st nt of the 3rd exon to the last nt of the 6th exon, evidenced a size ratio of exon *vs.* intron DNA of 1:4.15 *vs.* 1:4.45 in bovine counterpart. The different ratio observed between the two species is consequence of an artiodactyla retroposon (inverted Bov A element) located between nt 191 and nt 326 of intron 4 of bovine sequence (EMBL n° L19986). It is flanked by a 13 bp direct repeats (GGTAGCTGAGTCT), present as a single copy in the goat. Ramunno *et al.* (2004) recently showed that the goat *CSN1S1* gene is characterized by seven interspersed repeated elements *vs.* ten in the bovine one. Probably this extra retroposon element in the bovine *Lf* sequence is a rather young insertion and adds a further proof of the ancestral origin of the goat species as regards the bovine one, confirming that the retroposon insertions are a powerfull marker for phylogenetic studies of ruminants.

ACKNOWLEDGMENTS – This work was supported by Cofinanziamento Programmi di Rilevanza Nazionale (M.I.U.R.)

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