Identification of SNPs in the promoter of β-lactoglobulin gene in three Sicilian goat breeds

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ABSTRACT - The aim of this work was to sequence the full-length promoter region of the caprine β-lactoglobulin (β-lg) gene in three Sicilian goat breeds (Girgentana, Maltese, and Derivata di Siria), in order to identify polymorphisms, to search for transcription factors (TFs) sites, and to check if polymorphisms found lay within TFs binding sites. The promoter region of β-lg gene in Sicilian goat breeds showed high level of polymorphism due to the presence of 31 SNPs. Binding sites for several TFs were found within the goat β-lg promoter and within regions conserved between ovine and caprine species. Two SNPs were detected within TFs binding sites, such as MPBF and NF-I. Further studies are in progress to confirm polymorphic sites, to evaluate the possible effect of these mutations on binding affinity of TFs, their relationship with β-lg gene expression, and the functional role of SNPs within the TFs sites of the promoter region on milk traits.

Key words: β-lactoglobulin, Promoter, Goat, SNPs.

Introduction - β-lactoglobulin (β-lg) is the major whey protein in the milk of ruminants. It is also found in the milk of different mammalian species but it is lacking in humans (Brignon *et al.*, 1985; Monti *et al.*, 1989), rodents, and lagomorphs (Hambling *et al.*, 1992). Although no clear physiological functions have been defined for β-lg, a role in the transport of retinol and fatty acids has been suggested (Pérez and Calvo, 1995; Flower, 1996). However, the general affinity of β-lg with these hydrophobic molecules did not allow ascribing a specific role (Puyol *et al.*, 1991; Pérez and Calvo, 1995). Chianese *et al.* (2000) detected differences in β-lg content in milk of the Girgentana goat breed using HPLC analysis. Subsequently, Graziano *et al.* (2003) reported polymorphisms in the proximal promoter region (-341 T/C and -60 C/T) of different Italian goat breeds without identifying any correlation with β-lg content in milk. In 2005, Ballester *et al.* amplified and sequenced the β-lg gene in several goat breeds, including some individuals of the Girgentana breed. These authors found 15 SNPs, 9 of which were in the proximal promoter region and 6 in the exons. At present, no genetic variants affecting the amino acidic sequence of the caprine β-lg protein have been described at DNA level. The aim of this work was to sequence the full-length β-lg gene promoter region in samples of three Sicilian goat breeds (Girgentana, Maltese, and Derivata di Siria) in order to: i) identify polymorphisms; ii) search for transcription factors (TFs) sites; and iii) check if polymorphisms found lay within TFs binding sites.

Material and methods - In total, 9 samples of Girgentana, 10 of Maltese, and 8 of Derivata di Siria goat breeds were analyzed, and a set of 10 primers were designed. Primers BLG-F1 and BLG-R1 were used to amplify 2138 bp of the promoter region and 117 bp of exon 1 of β-lg gene (GenBank Acc.

no. Z33881). PCR reaction was performed in a final volume of 20 µl and the reaction mixture contained 1X PCR Master Mix, 10 µM of each primer, and approximately 75 ng of genomic DNA. The thermal cycling conditions were: 95°C for 5 min, 30 cycles of 95°C for 30 sec, 59°C for 1 min and 72°C for 1 min 30 sec, and a final extension of 72°C for 5 min. Primers BLG-F1 and BLG-R1 and other eight internal primers were used for sequencing reaction with BigDye v3.1 Cycle Sequencing Kit in an ABI PRISM 3130 Genetic Analyzer. SeqScape v3.1 software was used to analyze the nucleotide sequences, whereas Clustal W software (Thompson *et al.*, 1994) was used to align the sequences. TESS software (Schug and Overton, 1997) and AliBaba v2.1 software (Grabe, 2002) were used to predict binding sites for TFs, using the binding sites from TRANSFAC database (Wingender *et al.*, 1996). Only TFs identified with both software were considered.

Results and conclusions – The results of sequencing analysis and the alignment of the obtained sequences showed the presence on the whole of 31 different SNPs, 13 of which shared at least between two breeds. Six of these 31 SNPs confirmed the results reported by Ballester *et al.* (2005) that described these polymorphisms in several goat breeds including Girgentana samples. Considering the 31 SNPs, the Maltese breed presented the highest level of polymorphism with 19 SNPs, 9 of which were found only in this breed. The Girgentana and the Derivata di Siria breeds showed 14 SNPs each: in the Girgentana breed 7 specific SNPs were identified, whereas 2 SNPs were found only in the Derivata di Siria breed. Moreover, the three breeds shared 3 polymorphic sites: two transitions T/C in positions -642 and -2041, and one transversion G/C in position -980. In the analyzed samples, several potential binding sites for milk protein TFs were found within the first -700 bp of the β-lg promoter region (Table 1). The three binding sites, described in the ovine β-lg gene promoter (Watson *et al.*, 1991), and recognized by MPBF (Milk Protein Binding Factor) were well conserved in the caprine β-lg promoter. However, one mismatch was found within the region -93/-77 consisting in one G→T change. Three binding sites for NF-I (Nuclear Factor-I) were found in Sicilian goat breeds (Table 1), and no mismatches were present within the regions -159/-146, -271/-258, and -376/-363. Moreover, the binding site for NF-I in position -253/-240 was not present in goat promoter region because of 1 bp deletion in the first half of the consensus binding sequence of this element. The consensus site, for both MPBF and NF-I binding sites, was well conserved within the region -206/-197. However, the point mutations -205 C/T

Table 1. Transcription factors (TFs) within the first -700 bp of β-lg promoter region in Sicilian goat breeds compared with TFs in β-lg ovine promoter (Watson *et al.*, 1991).

	Ovine β-lg promoter		Caprine β-lg promoter	
TFs	Position	Sequence	Position	Sequence
MPBF	-93/-77	GATTCCGGGAACCGCGT	-93/-77	GATTCCGGGAACC**T**CGT
MPBF	-209/-197	TCTACCAGGAACC	-210/-198	TCTACCAGGAACC
MPBF	-277/-261	TGTTCCTGGGCACTGGCA	-277/-261	TGTTCCTGGGCACTGGCA
NF-I	-159/-146	TGGAAGAAGGCCTG	-159/-146	TGGAAGAAGGCCTG
NF-I	-253/-240	TGGACCCAGAGTCC	Not present	
NF-I	-271/-258	TGGCACTGGCAGCC	-271/-258	TGGCACTGGCAGCC
NF-I	-376/-363	TGGAGGAGCTGGTG	-376/-363	TGGAGGAGCTGGTG
NF-I/ MPBF	-205/-196	CCAGGAACCG	-206/-197	C**Y**AGGAACC**R**
MAF	-719/-711	GAGGGAAGT	-719/-711	GAGGGAAGT

(Y) and -197 G/A (R) were present in the region in which both factors are involved. Both mutations were found in the Maltese breed, the Derivata di Siria breed showed only the polymorphism in position -197, whereas the Girgentana breed did not present these SNPs. Moreover, the binding site for one MAF (Mammary cell-Activating Factor) was identified in the caprine promoter, in the region -719/-711, and an identical sequence was found in the ovine specie (Mink *et al.*, 1992).

It is well known that MPBFs are mammary gland-specific factors with an essential role in the regulation of milk protein gene expression, whereas NF-I could play a role in β-lg transcription (Watson *et al.*, 1991; Demmer *et al.*, 1995). Therefore, it should be important to determine if these SNPs could influence the binding affinity of TFs, and could affect the goat β-lg transcription level.

In conclusion, in the present work we reported a preliminary analysis of β-lg promoter polymorphisms in three goat breeds reared in Sicily, and new data on the comparison between caprine and ovine region of the gene were described. Further studies are in progress in a wider sample in order to confirm these SNPs, to evaluate the possible effect of these mutations on binding affinity of TFs, their relationship with β-lg gene expression, and the functional role of SNPs within the TFs sites of the goat β-lg promoter region on milk traits.

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