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Mapping of transcription factor binding Region of kappa casein (CSN3) gene in Iranian Bacterianus and Dromedaries camels

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ABSTRACT

κ-casein is a glycosilated protein in mammalian milk that plays an essential role in the milk micelles. Control of κ-casein expression reflects this essential role, although an understanding of the mechanisms involved lags behind that of the other milk protein genes. Transcriptional regulation, a first mechanism for controlling the development of organisms, is carried out by transcription factors binding sites (TFBSs). We determined the 5' region for Iranian Dromedaries and bactrianus camel κ-casein gene. We also determine their regulatory TFBSs regions. This region contained 14 potential TFBSs The four of them C/EBP-α, Oct1, MGF/STAT5 and TPB have almost perfectly conserved.

Key words: κ-casein, Bioinformatics analysis, Camels, Transcription factors

INTRODUCTION

Kappa casein (CSN3) is the milk protein that determines the size and specific function of milk micelles and its cleavage by chymosin is responsible for milk coagulation. (Yahyaoui et al,. 2003). Caseins are distinguished into four protein fractions, namely, alpha S1 (39-46% of total caseins), alpha S2 (8-11%), beta (25-35%), and kappa (8-20%) encoded by genes: CSN1S1, CSN2, CSN1S2 and CSN3 respectively (Farrell et al,. 2004). Variation in a genes promoter may change its expression or alter the amino acid sequence and affect the functional properties of the protein. κ-casein differs from other caseins in its solubility over a broad range of calciumion concentrations and contains a hydrophilic C-terminal region (Yahyaoui et al,. 2003). the results on association studies involving only coding region variants are not always consistent (Heck et al,. 2009) and this might be attributed to the presence of intragenic haplotypic combination of variants in the regulatory and noncoding regions (Prinzenberg et al,. 2003) Moreover, casein gene expression is also known to be differentially regulated by hormones and most of the potential hormone receptor binding sites occur within the 5′-flanking region of casein genes (Prinzenberg et al,. 2003). Thus, mutations at these regulatory regions might also have enduring effect on milk protein gene regulation at transcriptional level (Prinzenberg et al,. 2003 and Szymanowska et al,. 2004) either individually or as interior intragenic haplotypes. For CSN1S1, mutations in the promoter region have been reported to influence the protein-coding efficiency of the relevant structural gene by changing the binding affinity towards their respective nuclear transcription factors (TFs) (Malewski et al,. 1998 and M. Szymanowska et al,. 2004) and can thus be considered as functional candidate for milk protein content.

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Sequence variation within Alpha S1 5′-flanking region has been widely studied in several species like cattle involving mainly B. taurus (Prinzenberg et al,. 2005 and Kuss et al,. 2005) yak (Bai et al,. 2010), buffalo (Chianese et al,. 2009), goat (Ramunno et al,. 2004), and sheep (Bhure et al,. 2008). However, to date, very few studies are available on the non-coding region of kappa casein in camel. Further, due to evolutionary divergence, Iranian Bacterianus and Dromedaries are expected to have variations in the candidate genes related to dairy traits. Keeping in view the scanty information available Iranian Bacterianus and Dromedaries and other breed of camel, the present study was aimed to first: sequence the promoter of kappa casein; second: search for TFBSs region on Iranian Bacterianus and Dromedaries; third: identify homology in the regulatory domains for camel's kappa casein from different mammalian spaces.

MATERIALS AND MTHODS

Blood samples and DNA extraction

Blood samples were obtained from 5 Bacterian and 10 Dromedary camel randomly from Ardebil Camel Rearing Station and Mashhad Abattoir, respectively. Blood samples were immediately applied near the ice, was transported to the laboratory and stored until DNA isolation. DNA was extracted from 250 µl of blood, using a commercial kit (Bioneer Cat .NO .K-3032, USA) following the manufacturer's protocol. Restriction products were analyzed by electrophoresis on 0.8% agarose gel and the purity of the obtained DNA was verified by Nano Drop ND-2000 spectrophotometer (Thermo, USA).

PCR amplification

DNA regions (-1162 to +50 of CSN3 gene cluster) from the promoter region of CSN3 gene were amplified using the Personal Cycler™ thermo cycler (Biometra, Germany). The specific primers were designed by using Primer Premier 5, according to the available nucleotide sequences on the NCBI GenBank (EMBL ID HE863813) database and were synthesized commercially. The specific primers were as follows:

CSN3- F: 5′- TTGAAACTCTGCCATCTTTCTC-3′

CSN3- R: 5′- TGTGCCTGTCAGGTCTTGC-3′

PCR reactions were performed in a final volume of 25 µl with the reaction mixture containing 2.5 µl of 10X PCR buffer, 2 µl MgCl2 (mM) and 2 µl dNTPs (Mix), 1 µl of the DNA solution (50 to 100 ng/µl), 1.5 µl of mix primer (5 pmol/µl), 0.125 U/µl of *EX Taq* DNA polymerase (Takara, Japan), and some deionized water making up a final volume of 25 µl. PCR program was performed with an initial denaturation step at 94°C for 10 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec and extension at 72°C for 45 sec, and a final extension at 72°C for 10 min. The PCR correctness was assessed by electrophoresis of each sample (4 µl) on 0.8 % agarose gel and was sequenced by specific designed primers (Macro Gen, South Korea).

Bioinformatics tools

Then, SNP discovery, homology searches, comparison among sequences, and multiple alignments were accomplished using Basic Local Alignment Search Tool (BLAST) and CLC Main workbench version 5.5.

Interspersed elements were found by these two Web Server: (http://www.softberry.com/berry.phtml), (http://alggen.lsi.upc.edu/recerca/menu_recerca.html).

RESULTS AND DISCUSSION

The entire sequences (1212 bp) of the Iranian Bactrianus and Dromedaries CSN3 gene plus the analysis of 5′ flanking region was amplified. The accuracy of these fragments was visualized on using electrophoresis 0.8% agarose gel electrophoresis (Figure1).

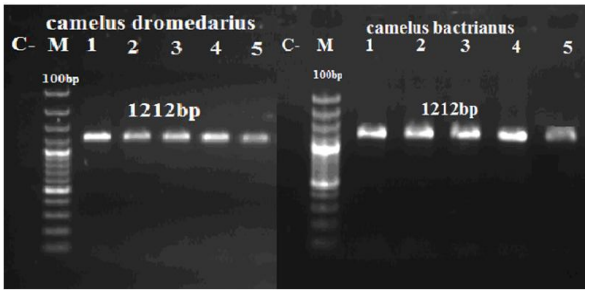
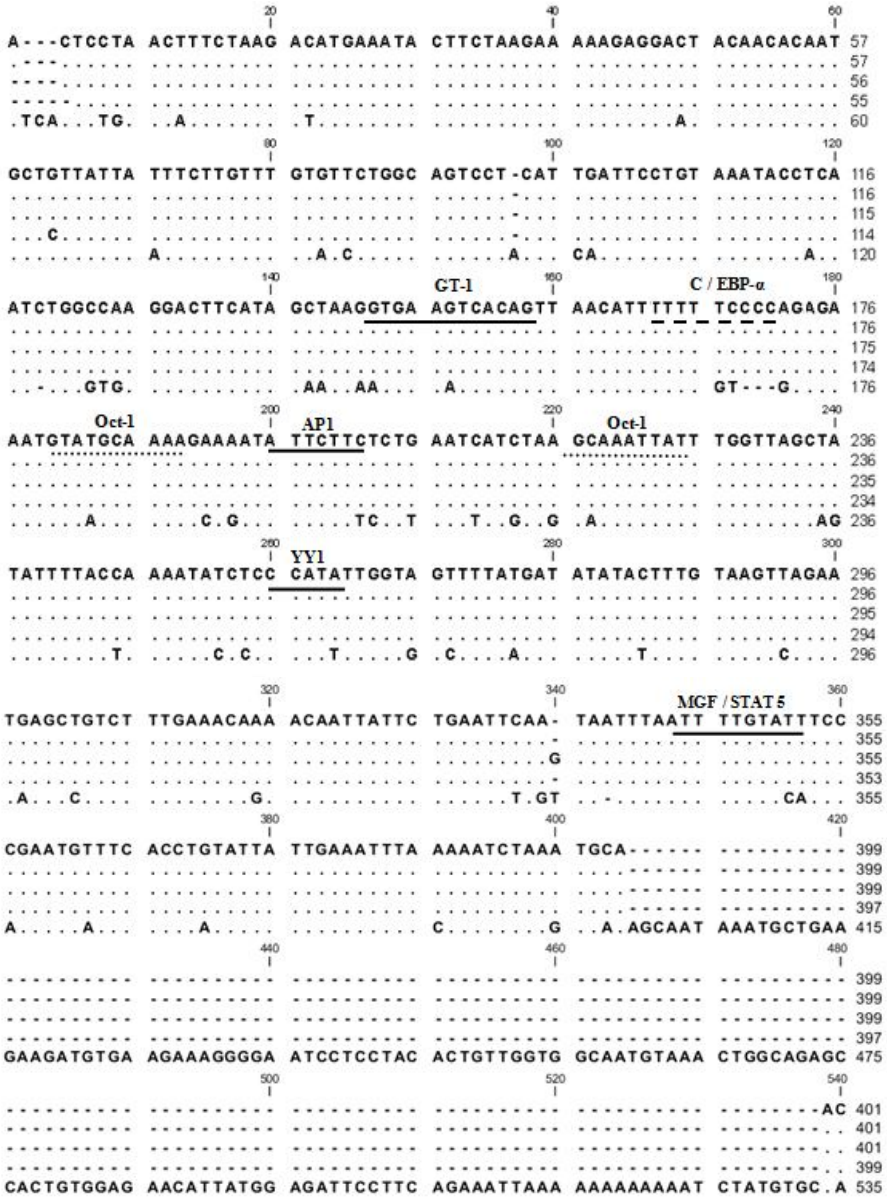


Figure1: PCR products of promoter region of CSN3gene on agarose 0.8%

Sequences have been published on NCBI gene database or GenBank under accession numbers: KJ755350 and KJ755349 for Dromedarius and Bactrianus, respectively.

A homology and similar locations of most putative transcription binding sites were already reported among ovine, caprine, bovine, murine and rabbit κ-casein promoters (Gerencser et al., 2002), horse, donkey and zebra CSN3 5' flanking region (Hobor et al., 2008) C.dromedarius, C.bactrianus, E.asinus, B.taurus CSN3 5' flanking region (Pauciullo et al, 2013). In this work we performed a comparative analysis between two sequence that obtain in this work with two published sequences of the camels (EMBL ID: HE863813; AJ409280) and B.taurus (EMBL ID: AY380228).

Using manual and bioinformatics searches, 14 potential TFBSs were identified within about 1050 bp of the CSN3 gene promoter region. While, Pauciullo et al, 2013 report 25 putative consensus sequences for transcription factor binding sites in their study, that some of this region are similar with present work. The TATA-box location was performed by using analysis the available sequences were identified (figure1).


GT-1
C / EBP-α
Oct-1
AP1
Oct-1
YY1
MGF / STAT5

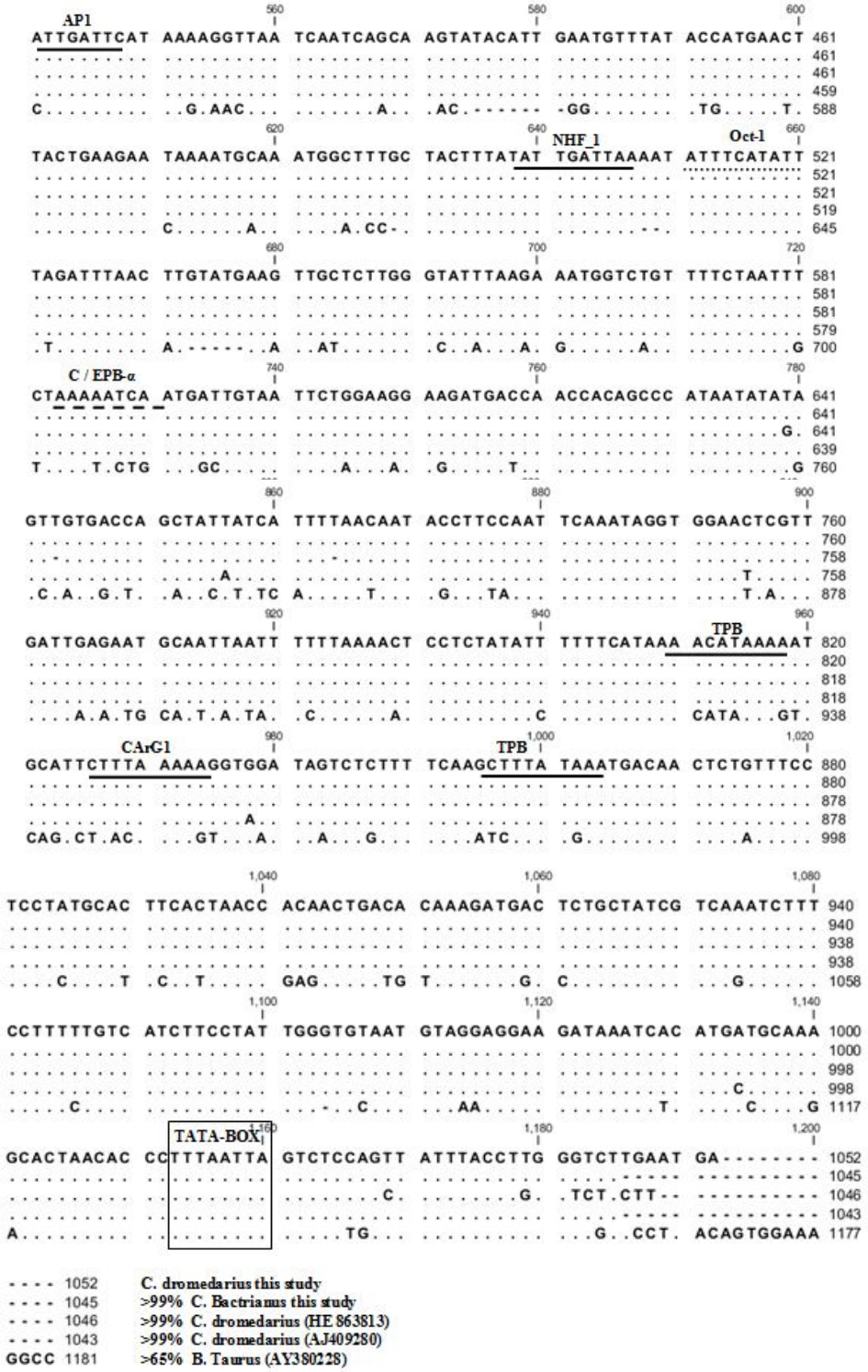


Figure1. Alignment between the nucleotide sequences of the promoter region dromedarius and bactrianus CSN3 gene with the published sequences of the camels (EMBL ID: AJ409280, HE863813) and cattle (EMBL ID: AY380228) sequences. The boxes represent show TATA-box.

The initial and final regions (approximately 200, 900-1050 bp) upstream of the transcription start site was found to be the least conserved within the entire promoter. The adjacent upstream region of

approximately 800 bp contained form 14 TFBSs that found, 4 putative transcription factor binding sites almost perfectly conserved: three Octamer bind protein (Oct-1), one Mammary Gland Factor/STAT5 (MGF/STAT5), two enhancer bind protein (C/EBP-α), and two Tata Binding Protein (TBP). The MGF/STAT5 and (C/EBP-α) sites were reported to be very well conserved in more than 9 species (Pauciullo et al., 2013; Gerencser et al., 2002; Hobor et al., 2008; Malewski, 1998). Here confirm them also in camels increasing the probability that these factors are necessary for the regulation of the gene expression (Pauciullo et al., 2013). It interacts directly with STAT 5, reported as a crucial functional activator of casein gene expression (Vanselow et al., 2006). Oct-1 is not known to be a strong transcriptional activator by itself, but in conjunction with other co-activators. For instance, Oct-1 and STAT5 are both involved in the hormonal induction of casein gene expression (Zhao et al. 2002). They bind DNA cooperatively and they interact in the stabilization of the transcription complex. However, Oct-1 can also interact with the TBP components (Zwilling et al., 1994). Yin Yang 1 (YY1) activator protein 1 (AP1) were conserved in equids (Pauciullo et al., 2013; Hobor et al., 2008). Nuclear factor 1 (NF1) conserved among primates and equids (Pauciullo et al., 2013; Hobor et al., 2008). This site found in the camel promoter were identied as common motifs in 28 milk protein gene (Akos et al,. 2001). For GT1 and CArG1 are not available reports prior to this study.

The table1 shows their location, and sequences of this TFBSs.

Table1. Occurrence of putative transcriptions factor binding sites in the 5' region of Iranian dromedaries and bactrianus camel κ-casein. Positions are relative to the TATA boxes

Factor	Iranian dromedarius and bactrianus camel
GT_1	-1000: AAG**GTGAAGTCACAG**TTA
C / EBP-α	-978: ATT**TTTTTTCCCC**AGA
	-422: TCT**AAAAATCA**ATG
Oct-1	-963: AAT**GTATACA**AAA
	-923: TAA**GCAAATTAT**TTG
	-493: AAT**ATTTCATATT**TAG
AP1	-946: AAT**ATTCTTC**TCT
	-604: GCA**ATTGATTC**ATA
MGF / STAT5	-796: TTA**ATTTTGTATT**TCC
NHF-1	-507: TAT**TATTGATTAA**AAT
TPB	-195: ATA**AAACATAAAA**AAT
	-147: CAA**GCTTTATAAA**TGA
CArG1	-180: ATT**CTTTAAAAG**GTG
YY1	-888: AGC**CCATA**ATA

CONCLUSION

We performed a genome-wide search transcription factor binding sites in Iranian dromedaries and bactrianus camel and identified a total of 14 TFBSs. The distribution of these regulatory motifs along promoter region sequences, which allowed us to accurately detect DNA regulatory

sequences that influence gene expression. Our work greatly expanded our knowledge of regulatory sites in camel and is a valuable step toward building a genome-wide regulatory network of camel.

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