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MOLECULAR CHARACTERIZATION OF THE B- AND K-CASEIN GENES (*CSN2* AND *CSN3*) IN DROMEDARY CAMELS

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Abstract

The β-casein is the most abundant component of the micelle in camel milk,whereas the κ-casein plays a fundamental role for its stabilization.In the present study,we report the characterization at molecular level of the β- and k-casein genes (*CSN2* and *CSN3*) in dromedary camels.The *CSN2* is spread over 7.8kb and consists of 9 exons,from 24bp (exon 5) to 519bp (exon 7),and 8 introns from 95bp (intron 5) to 1950bp (intron 1).The composite response element (CoRE) region was identified in the promoter,whereas the presence of mature microRNAs sequences improves the knowledge on the factors involved in the gene regulation.46 SNPs have been detected.The transition g.2126A>G falls within the TATA-box with a putative influence on the transcription.Five interspersed repeats were identified, whereas the presence of putative bio-functional peptides confirms the potential protective role of the camel milk for the human nutrition.The *CSN3* covers 9.3kb and consists of 5 exons,from 33bp (exon 3) to 494bp (exon 4),and 4 introns from 1200bp (intron 3) to 2928bp (intron 2).The regulatory regions of camel κ-casein seems to be more related to equids than to other species.17 SNPs have been detected.The SNP g.1029T>C is responsible for the creation of a new putative sequence for the transcription factor HNF-1.

Key words: β-casein; κ-casein; CSN2; CSN3; dromedary camel.

МОЛЕКУЛЯРНЫЕ ХАРАКТЕРИСТИКА В- И К-КАЗЕИН ГЕНОВ (CSN2 И CSN3) У ВЕРБЛЮДОВ ДРОМЕДАРОВ

β-казеин является наиболее распространенным компонентом мицеллы в верблюжьем молоке, где κ-казеин играет фундаментальную роль для их стабилизирования. В этой работе мы докладываем описание генов β- и k-вазеина на молекулярном уроване (CSN2 и CSN3) у верблюдов дромедаров. CSN2 распространяется на 7.8 кб и состоит из 9 экзонов, от 24 бп (экзон 5) до 519 бп (эксон 7), и 8 интронов от 95 бп (интрон 5) до 1950 бп (интрон 1). Область составного элемента ответа (CoRE) была определена в промоутере, где присутствие зрелых последовательностей microRNAs улучшают знания о факторах участвующих в регуляциях генов. Были обнаружены 46 SNPs. Переход g.2126A>G подпадает с TATA-ящиком с предпологаемым влиянием на транскрипцию. Были обнаружены пять перемешанных повторов, где присутствие предполагаемых био-функциональных пептидов подтверждают потенциальную защитную роль верблюжьего молока для потребления человеком. CSN3 покрывает 9.3 кб и состоит из 5 эксонов от 33 бп (эксон 3) до 494 бп (эксон 4), и 4 интронов от 1200 бп (интрон 3) до 2928 бп (интрон 2). Регулирующие κ-казеин регионы верблюдов кажутся более связанными с equids чем с другими видами. Были обнаружены 17 SNPs. SNP g.1029T>C отвечает за образование новых предпологаемых последовательностей для транскрипции факторов HNF-1.

Ключевые слова: β-казеин; κ-казеин; CSN2; CSN3; верблюды дромедары.

Introduction

As for the other mammals, the main component of camel milk proteins are caseins, among which the β-casein (β-CN) is the most abundant component (~65%) (Kappeler et al. 2003). This amount is definitively higher than the 45% reported in bovine milk (Farrell et al., 2004). On the contrary, the camel κ-casein (κ-CN) showed significantly lower amounts compared to the homologous cow's casein (Kappeler et al. 2003). In ruminants, the β-CN plays an essential role both for the nutrition aspects and for the impact on the technological properties of the milk and dairy products. In fact, the occurrence of different levels of phosphorylation are reported to affect the distribution of calcium (Amigo et al. 2000). Conversely, the κ-CN plays an essential role in the casein micelle stabilization being the specific substrate of the chymosin, responsible for the hydrolyzation of the κ-CN into the para-κ-CN and the caseino-macropeptide (CMP).

Caseins (αs1, β, αs2 and κ) are coded by single autosomal genes (*CSN1S1, CSN2, CSN1S2* and *CSN3*, respectively) clustered in a DNA stretch of about 250 kb. They have been deeply studied in ruminants and very well characterized both at DNA and protein level. For instance in cattle at least 17 alleles corresponding to 12 protein variants have been identified for the β-CN (Caroli et al. 2009) and at least 19 alleles corresponding to 14 variants have been found for the κ-CN (Caroli et al. 2009). Conversely, in camels no deep investigation has been reported so far. The cDNA sequences for all caseins were reported by Kappeler et al. (1998), while a partial genomic DNA sequence for αs1-CN was reported by Shuiep et al. (2013). Considering the growing interest that camel milk is globally receiving especially for the potential health benefits obtained through a number of bioactive components of camel milk, a deep investigation was undertaken to characterize the *CSN2* and *CSN3* genes in dromedary camels and to explore the genetic variability at these loci.

Materials and methods

Blood samples were collected from 188 Sudanese dromedary camels reared in five regions of the country and belonging to different ecotypes including Shanbali, Kahli, Lahaoi and Arabi camels. DNA was isolated by Spin Blood Mini Kit (Invitek, Germany). Four test samples (one for each ecotype) were chosen for the sequencing of the whole camel *CSN2* and *CSN3* gene. A set of 31 primers for β-CN and 33 for κ-CN were designed for amplification and sequencing by DNAsis-Max software (Hitachi). Polymerase chain reaction (PCR) was used to amplify the gene fragments. Amplicons were then purified and sequenced by ABI 3100 Genetic Analyzer using BigDye chemistry (Applied Biosystems). SNP discovery and multiple alignments were accomplished using DNAsis-Max software (Hitachi). The putative transcription factors were searched by Transfact 7.0 software. Interspersed elements were found by RepeatMasker (http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker). Potential microRNA sequences were discoverd by the bovine miRBase database (http://www.mirbase.org/search.shtml).

Results and discussion

The *CSN2* and *CSN3* genes were completely sequenced and characterized in dromedary camels. The *CSN2* is spread over 7.8 kb and consists of 9 exons, ranging in size from 24 bp (exon 5) to 519 bp (exon 7), and 8 introns, ranging from 95 bp (intron 5) to 1950 bp (intron 1). The *CSN3* is spread over 9.3 kb and consists of 5 exons, ranging in size from 33 bp (exon 3) to 494 bp (exon 4), and 4 introns, ranging from 1200 bp (intron 3) to 2928 bp (intron 2). On the whole, they share a similar organization compared to the homologous genes in other species. However, some differences in intronic size characterize camel sequences. The ratio exon/intron is slightly higher (1:6.01) for the β-CN and higher (1:10.41) for the κ-CN when compared with the corresponding bovine gene(s) sequences (1:6.74 and 1:14.42 for β- and κ-CN respectively). Such difference in size is also due to a different number of interspersed elements: 5 in camel *vs* 9 in bovine for β-CN; and 7 *vs* 10 for k-CN. Two LINEs appears to be characteristic of the camel sequences, since they have not been found by the comparison with bovine, buffalo, donkey, and human β- and κ-casein genes. Therefore, they could be used as genetic marker typical of the specie, as well as for evolutionary and clustering studies.

A total of 302 high-scoring (85%-100%) putative binding sites were found by TFSERCH tool. Most of the consensus sequences related to protein and milk production (47 in total for the β-CN and 25 for the κ-CN) were identified in the proximal promoter region. The close proximity of STAT5, GR and C/EBP-β between -900/-690bp probably represent the camel β-CN composite response element (CoRE). These elements are known to be transducers and activators of transcription (Lechner et al. 1997). Oct-1 and TBP instead are very representative motifs of camel κ-CN gene promoter. A comparative analysis with the 5'-flanking region reported for the ovine, caprine, bovine, murine, rabbit, horse, donkey, zebra, buffalo and human k-CN showed that camels and equids have a slightly lower level of divergence (~20.8%) compared with ruminants (≤26.6%). Phylogenetic data confirmed that camels belong to their own branch, but its κ-CN promoter appears more related to equids (homology >80%) than to the other species (similarity ~60%).

63 SNPs (46 for the β-CN and 17 for the κ-CN) have been found in total by the comparison of our sequences with the promoters and cDNAs already available in database. In particular the SNP g.2126A>G detected in dromedary *CSN2* promoter falls 3 bp downstream the TATA-box, changing the binding affinity from 87.7% to 86.2%. Conversely, the g.1029T>C SNP, located in the promoter region of the *CSN3*, creates an extra putative site for the transcription factor HNF-1 which might be a potential regulator of casein genes expression. 17 putative mature sequences for microRNA were found in camel *CSN2*. Six out 17 are located in the intron 3 and they recognize almost the same sequence in a stretch of DNA of 20 bp. The biological functions of most miRNA are unknown, but it is estimated that >30% of protein-coding genes are regulated by miRNA (Lewis et al., 2005). For instance, one of the target genes for miR-190a and miR-2437 is C/EBP-α whose transcription factor binding site is present in the proximal promoter region of camel *CSN2* and therefore these miRNAs might be regulator of the gene expression.

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