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## Short Communication

# 5′ Flanking Region of Kappa-Casein Gene is Highly Polymorphic in Eight Dromedary Camel Breeds of Pakistan

**Masroor Ellahi Babar***

*Department of Molecular Biology, Virtual University of Pakistan, Lahore, Pakistan.*

**ABSTRACT**

Kappa-casein played critical role in the expression of milk protein. It stabilizes the milk micelle and gene 5' flanking region which works valuably in transcription regulation. Current study illustrates that Kappa-casein gene 5˝flanking region in Dromedary camel of Pakistan is highly polymorphic and it is phylogeneticaly linked with other mammals. The analyses of the sequence of 5˝flanking region in various breeds reveals the presence of different polymorphic regions includes cysteine nucleotide deletion, AT bases insertion and transvertions (C>G; G>T). Deletion of A bases at c.1-1075 and hetrozygosity (CT) at the c1-1046 were analyzed along with some other SNPs are very important. These evidences points towards the change of expression of those protein which are nearest to the region of gene regulation and enhancer region. After observing taxas several distinct clads of camel breeds it was found that Kharani breed showed the close relationship with the *C. dromedarius* camel and all the breeds show a common ancestor that is sheep. For Casein loci in the Pakistani breeds these SNPs are the first to be reported.

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Camel is a species of unique characteristics which enable them to adopt and survive in harsh desert environment and also economically and historically very important species throughout the world particularly in the Asia and Africa (Schwartz, 1992; Nagarajan *et al*., 2012; Pauciullo *et al*., 2013; Sabahat *et al*., 2020) Milk from camel is very important in arid and semi-arid regions throughout the world for the survival of Pastoral, Nomad and Bedouin households and after the domestication of camel a huge population of migrants are being supported worldwide. Camel of Dromedary species is much important domestically than the other 5 species of family camelidae (*Camelus bactrianus*, *Lama Guanicoe*, *Lama Glama*, *Vicugnavicugna and* Vicugnapacos). During the lactation period of twelve to eighteen months, 3 to 10 kg per day milk production is estimated by dromedary camel (Farah *et al*., 2007). The milk contains about 3.1% fat and 2.9% protein depending upon the management conditions, feeding, breed and lactation stage (Al haj and Al Kanhal, 2011). Due to high amount of casein as chief constituent of camel's milk protein (Sawaya *et al*., 1984), the milk of camel is significantly have higher nutritive value than other milking animals milk (Barłowska *et al*., 2011). The fraction of casein is about 52 - 89% (Al haj and Al Kanhal, 2011; Ereifej *et al*., 2011) and further divided into 4 fractions i.e. k-CN, b-CN as1- and as2 (Ochirkhuyag, *et al*., 1997; El-Agamy, 2007), which are encoded by 4genes, CSN3, CSN2 CSN1S1 and CSN1S2, respectively (Kappeler *et al*., 1998). In the milk of camel, the chief fractions are (65%) b-CN, (22%) as1-CN and (3.5%) k-CN respectively (El-Agamy, 2007). The polymorphic sites are present in these casein proteins related genome sequence in the species of the most animals. The function of protein in the milk of camel could be articulated by using different electrophoretic methods or chromatographic like isoelectric focusing (IEF) or SDS-PAGE (Alim *et al*., 2005; Zhang *et al*., 2005; Ereifej *et al*., 2011).

However, in Pakistan camel breeds inconsistency in the milk protein level is hardly described. Globally increasing interest rate of camel's milk is because valuable benefits could be gained through it. So, the current study was simulated to expose the polymorphism in 5˝flanking region and genetic relatedness with other mammals of Kappa-casein gene in Pakistani dromedary camel.

*Materials and methods*

Blood samples were collected from Cholistan area of Pakistan in vaccutainers from different camel breads including Bareela, Pahri, Mareecha, Thari, Watni, Kachi, Kharani and mix breed and studied. Manual method were used to extract genomic DNA following the method of (Sambrook and Russell, 2001) from each sample and

* Corresponding author: masroor.ellahi@vu.edu.pk
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concentrations were brought to the same level 50 mg/µl.

Amplification of purified extracted DNA was done using kappa-casein gene 5 flanking region. designing of primers was carried out by primer designing software known as primer3 software (Table I) and NCBI was used as source for the targeted gene sequence (Accession no. AJ409280.1). PCR amplification methods included following conditions such as initial denaturation at 95°C (5 min), primer annealing temperature was adjusted according to the (Table I), extension of the gene was performed at 72°C for 45 seconds and final extension was carried out at 72°C for 10 min. Then PCR product was sent for sequencing with ABI 3100 genetic (Applied Biosystem) sequence analyzer.

Sequenced data was manually aligned and analyzed. MEGA 6 software package was used for the construction of phylogenetic tree (Kumar *et al*., 2016).

**Table I. Kappa-casein gene 5' flanking region primer list.**

| Primer Name | Product Size (bp) | Primer sequence |
|---|---|---|
| k-casein3F | 390 | AGTGTGTGACCAGCTATTATCA |
| k-casein3R | | AGGTCTTGCTTGGCAGTAG |
| k-casein1F | 459 | CTGAACAGCAGAAGCCAACT |
| k-casein1R | | AGGTGAAACATTCGGGAAAT |
| k-casein2F | 598 | CCCCAGAGAAATGTATGCAA |
| k-casein2R | | TCAATCAACGAGTTCCACCT |

*Results and discussion*

Phosphoproteins are the main important constituent of mammalian milk and Kappa-casein belong to this phosphoproteins family, also Kappa casein is a glycosilated protein, (as1, β, as2, κ). An important role is played by Kappa-casein for the calculation of specific function and size in caseinmicelle stabilization. The examination of 5 flanking region of Kappa casein provides us very useful information about the transcription analysis factors. About the expression regulation of gene this analysis is very helpful. Mutation of any type in this particular patch, change the mRNA stability or modify the transcription rat (Rijnkels, 1998). So, this preceded to the alteration in the components of specific protein of milk. In the current study, to examine the presumed regulatory regions among various breeds of dromederius camel sequencing of 5 flanking region Kappa-casein gene was conducted for SNP discovery. In the upstream position of Kappa-casein gene, various polymorphic positions were observed as shown in the Table II.

Various single nucleotide substitutions, transversions (C>G, G>T), transition (A>G), deletion and insertion of nucleotide were observed. Transversions of G>T at the position 1578 has been described for the milk and yield of milk protein also in Holstein cattle. Genetic variations in camel milk protein alpha casein have also been reported (Shuiep *et al*., 2013). Kappeler *et al*. (1998) has described non-allelic variants of similar fraction of casein as an exchange of amino acid. Pauciullo *et al*. (2013) have been recently reported 17 SNPs (polymorphic sites) in camels and also analyzed CSN3 Kappa-casein gene. By using the available reference sequences of species including; Capra hericus, Bostaurus, Ovisaries, Camelusdromedarious and, a polygenetic tree of studied k-casein gene of camelbreeds from Pakistan was formulated to see the genetic accordance with them. Phylogenetic tree is shown in Figure 1.

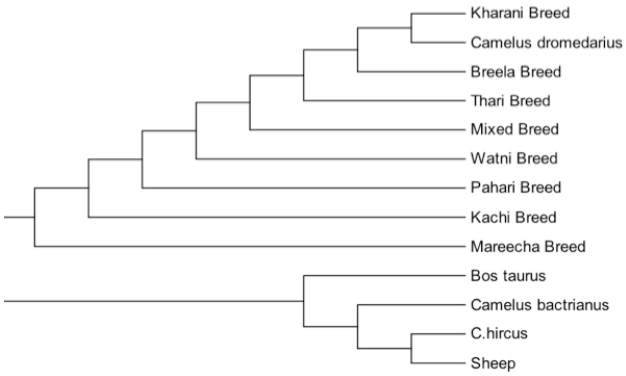


Fig.1. Evolutionary relationship of camel bread.

In the cattle polymorphism in the κ-casein was described well by (Prinzenberg *et al*., 2008) sheep (Ceriotti *et al*., 2004; Caroli *et al*., 2009) and goat (Jann *et al*., 2004; Prinzenberg *et al*., 2005). In CSN3 gene cows and Goats express most allelic polymorphism than the other farm animals (Ramunno *et al*., 2004; Caroli *et al*., 2009). Yahyaoui *et al*. (2003) described that the solubility of casein was affected by allelic polymorphism in κ-casein gene C-terminal region However, the analysis of coding region sequence of κ-casein in association studies is not always reliable (Heck *et al*., 2009) and might be attributed to the existence of intragenic haplotypic variants combination in the noncoding and regulatory regions (Prinzenberg *et al*., 2003).

Kappeler *et al*. (2003) examined the close association with different species by describing the 5 flanking regions of milk gene for camel milk. 5'-upstream sequences arrangement of protein genes for camel milk was closely associated with their analogous complements from the other animal species. In the mammary gland, these are

**Table II. Kappa casein gene 5' flanking region polymorphic variations different breads of camel of Pakistan.**

| Sr# | Position | Sequence change | Type of change | Sr # | Position | Sequence change | Type of change |
|---|---|---|---|---|---|---|---|
| **Kachi breed** | | | | **Watni breed** | | | |
| 1 | c.1-1075 | del. A | Deletion | 1 | c.1-1075 | del. A | Deletion |
| 2 | c.1-1046 | GT | Heterozygous | 2 | c.1-1046 | GT | Heterozygous |
| 3 | c.1-707 | del. G | Deletion | 3 | c.1-982 | GC | Heterozygous |
| 4 | c.1-684 | A>T | Transversion | 4 | c.1-707 | del. G | Deletion |
| 5 | c.1-683 | T>G | Transversion | 5 | c.1-1156 | AT | Insertion |
| 6 | c.1-402 | Ins. T | Insertion | | | | |
| 7 | c.1-340 | Ins. T | Insertion | **Kharani breed** | | | |
| 8 | c.1-321 | Ins. T | Insertion | 1 | c.1-1046 | GT | Heterozygous |
| 9 | c.1-1085 | del. A | Deletion | 2 | c.1-982 | GC | Heterozygous |
| 10 | c.1-1081 | Ins. A | Insertion | 3 | c.1-707 | del. G | Deletion |
| 11 | c.1-1036 | A>G | Transition | | | | |
| 12 | c.1-1035 | A>G | Transition | **Thari breed** | | | |
| 13 | c.1-682 | del. G | Deletion | 1 | c.1-707 | del. G | Deletion |
| 14 | c.1-525 | GA | Heterozygous | | | | |
| **Mareecha breed** | | | | **Pahari breed** | | | |
| 1 | c.1-1075 | del. A | Deletion | 1 | c.1-1075 | del. A | Deletion |
| 2 | c.1-1046 | GT | Heterozygous | 2 | c.1-1046 | GT | Heterozygous |
| 3 | c.1-982 | GC | Heterozygous | 3 | c.1-982 | GC | Heterozygous |
| 4 | c.1-707 | del. G | Deletion | 4 | c.1-707 | del. G | Deletion |
| 5 | c.1-684 | A>T | Transversion | 5 | c.1-684 | A>T | Transversion |
| 6 | c.1-683 | T>G | Transversion | 6 | c.1-683 | T>G | Transversion |
| 7 | c.1-1088 | G>T | Transversion | 7 | C1-1057 | ins T | Insertion |
| 8 | c.1-1087 | A>G | Transition | | | | |
| 9 | c.1-1054 | C>T | Transition | | | | |
| **Mixed Breed 3500-4000** | | | | **Breela Breed** | | | |
| 1 | c.1-1075 | del. A | Deletion | 1 | c.1-1075 | del. A | Deletion |
| 2 | c.1-1046 | GT | Heterozygous | 2 | c.1-1046 | GT | Heterozygous |
| 3 | c.1-982 | GC | Heterozygous | 3 | c.1-982 | GC | Heterozygous |
| 4 | c.1-707 | del. G | Deletion | 4 | c.1-707 | del. G | Deletion |
| 5 | c.1-684 | A>T | Transversion | 5 | c.1-684 | A>T | Transversion |
| 6 | c.1-683 | T>G | Transversion | 6 | c.1-1081 | ins. A | insertion |
| | | | | 7 | c.1-690 | ins. G | insertion |

gene expression level independent in the other species. An interesting fact about the 5'-upstream sequences of camels is to show the significant relatedness in the conditions in which the particular genes were delineate to express a fantastically different level in various specie's lactating mammary glands e.g. k-CN in case of camel and bovine (Kappeler *et al*., 1998). This sequence relatedness stipulate that the modulation of gene expression has to be regulate to little perpetuated areas within the 5'-upstream sequence. Mutations in these areas will commence, eradicate or modify the tissue and stage specific proclamation of milk protein genes.

Kappa-casein is considered as an important biomarker for milk production in dairy animals. On comparing different camel breeds, kappa-casein gene is observed to be very polymorphic from upstream region and could

prove to be a proficient molecular marker in selection. As not enough information is available so the polymorphism found in in the current study in the casein cluster in camels can be used as the first genetic marker in the arrangement of the 5'-upstream sequence. Data collected from different breeds of camels in the current study which indicated polymorphic sites would promote practicability for a quick directional selection in esteem of such allele.

*Statement of conflict of interest*

The authors declare there is no conflict of interest.

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