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Short Communication

SEQUENCE DIVERSITY OF BoLA-DRB3 GENE IN PAKISTANI DROMEDARY CAMEL

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ABSTRACT

BoLA-DRB3 gene is responsible for the differences in the susceptibility to infectious disease in mammals and is considered more-appropriate for comparative evolutionary studies. Here we present 247 bp sequence of BoLA-DRB3 gene exon 2 in 20 individuals of Mareecha camel breed and non-descript camels of Pakistan. We identified this gene highly polymorphic as 10 different polymorphic sites (3 singleton and 7 Parsimony informative sites) were detected that may be related to the variability in the immune responsiveness of different individuals to particular pathogens. Ten identified haplotypes showed haplotype diversity 0.879 and nucleotide diversity 0.01445. The phylogenetic tree was also constructed using *MEGA ver*. 5.05 which indicated close relationship between camel and *Ovis aries*. This is the preliminary report on MHC genes in Pakistani camel that may be useful for the association of polymorphisms with the disease resistance and tolerance to various infectious agents in different breeds of camel in future.

Key words: BolA-DRB3 exon 2, Sequences, High polymorphism, Pakistani camel.

INTRODUCTION

Pakistan is Animal Genetic Resource (AnGR) rich country having different breeds of camel with population of more than 1 million being third among major camel raising countries in the world. All native camel breeds of Pakistan are one-humped camel (*Camelus dromedarious*). The camel has been serving millions of people from arid, semi arid and desert areas of world from various centuries. Camel with its unique physiological features serves as source of food, fiber, transportation and social status to people in world as well as to marginalized people in Pakistan. In spite of rich diversity of camel breeds in Pakistan, very little work is done on Pakistani camel to understand the genetic architecture and relationship among different camel breeds.

In cattle MHC is termed bovine leukocyte antigen (BoLA) (Andersson *et al*., 1988). Major histo-compatibility complex (MHC) genes (immune genes) are considered under strong pathogen-driven selective forces. The mammalian MHC is divided into class I and II genes (Hughes and Yeager, 1998).

There is substantial work done on the major histocompatibility complex (MHC) class II genes to understand the level of polymorphism in different mammals although a limited work have been done on camel DRB3 gene. The DRB3 gene (that encodes β1 domain, responsible for peptide-binding sites) is the most polymorphic of the cattle MHC genes (Bhel *et al*, 2007). MHC Class II genes play an important role in the genetic control of immune responses. The main MHC class II restriction elements are products of the DRB and DQA genes (Lewin *et al*., 1999). Among DRB, the DRB3 exon 2 has been extensively studied and exhibits highly polymorphism with more than100 alleles (Poli *et al*., 2003; Miltiadou *et al*., 2003).

MATERIALS AND METHODS

Pure animals of Mareecha camel breed (n=10) and non-descript camel (n=10) from were selected from Camel Breeding & Research Station, Rakh Mahni, Bhakkar, Punjab and Mithra Bangla Tehsil. Yazman District Bahawalpur, Punjab. Ten mL blood sample was collected from Jugular vein in EDTA added tubes and placed on ice and shifted to Molecular Biology and Genomics Lab of Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore for DNA extraction following protocol devised by Hussain *et al.*, 2013. DNA samples were run on 0.8% Agarose gel along with standard for quantification. The concentration of all samples was adjusted to 50 ng/uL to be used as template in PCR. Specific primers DRB3-F 5'-TATCCCGTCTCTGCAGCACA-3' and DRB3-R 5'-TCGCCGCTGCACACTGAAAC-3' were designed for the amplification of DRB3 gene exon 2 from *Bos taurus* sequence available in GenBank NCBI (Accession no AB523809) using the Primer3 software v. 0.4.0 (Rozen and Skaletsky, 2000). The exon 2 of DRB3 gene was amplified by using a reaction mixture of 25 µL with 1µL of template DNA, 2.5 mM $MgCl_2$, 100 µM of dNTPs mix, 0.1µM of each primer and 1.5 U of Taq DNA polymerase (Fermentas, Thermo Fisher Scientific Inc. USA). The PCR was carried out in BioRad thermocycler using initial denaturation of 94ºC for 5 min, 35 cycles of

denaturation at 94ºC for 30s, annealing at 60 °C for 30s and extension at 72°C for 30s followed by final extension at 72ºC for 7 min. PCR products were sequenced with the help of ABI prism Genetic Analyzer 3130 XL (Applied Biosystems, Inc., Foster City, CA). Sequence results were cleaned and SNPs were identified with the help of CodonCode Aligner software (v. 4.0.4, Codon Code Corporation). DnaSP software (Librado, P. and Rozas, 2009) was used to find polymorphic sites, number of haplotypes, haplotype and nucleotide diversity. Phylogenetic analysis was done using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) through MEGA software (V 5.05, Tamura *et al*., 2011) was used with 1000 bootstrap value.

RESULTS AND DISCUSSION

- The finally selected 247 bp portion of DRB3 exon 2 showed polymorphisms at 10 different positions. There were 3 singleton variable sites (at position 177, 198, 222) and 7 Parsimony informative sites at position 64, 135, 143, 174, 187, 197, 199. Ten haplotypes were found with 0.879 haplotype diversity. While nucleotide diversity was 0.01445. The Phylogenetic analysis (accession no of different mammals shown in the tree) showed that all identified haplotypes are clustered together and closer to *Ovis aries*. *Bos grunniens* also positioned close to camel. *Bubalus bubalis*, *Capra hircus* and deer fall in separate branches close to camel. *Gorilla gorilla* and *Homo sapiens* share the same clade. Sea lion, bottlenose dolphin, domestic cat, dog and pig fall near to each other and rat and mouse share the same clade. *Bos taurus* and *Bos indicus* were used as outer group and they are very well separated from camel DRB3 exon 2 genome showing high genetic difference between them. The tree reconfirmed the status of other *Bovidae*, *Canidae and Felidae* families. *Bos taurus* and *Bos indicus* was grouped together while *Bubalus bubalis*, *Bos grunniens*, *Capra hircus* and deer kept their separate branch. Gorilla and *Homo sapiens* were also grouped together. The DRB3 gene was found very useful for inferring variation in immune responses as well as the evolutionary understanding in different species. This study provided new information to examine the MHC-pathogen co-evolution in other camel breeds as well.

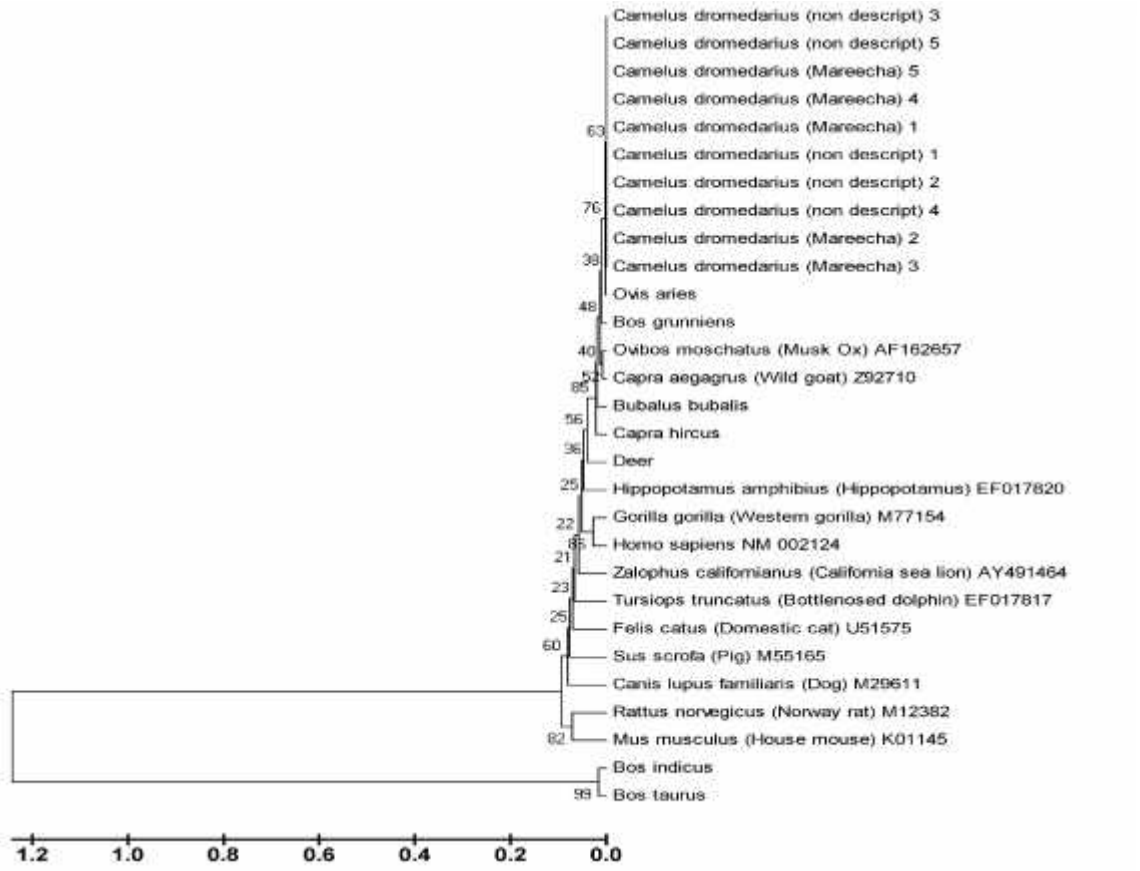

Fig. 1. Phylogenetic tree (UPGMA method, using MEGA 5.05) of Pakistani camel haplotypes constructed with DRB3.2 sequences of other mammals reported in GenBank. The bootstrap support values are indicated on each node.

Conclusion: Among MHC class II, DRB3 exon 2 is very polymorphic in studied Pakistan camel breeds. The Phylogenetic analysis also revealed the unique position of camel among other mammals. This first report on camel from Pakistan will serve as baseline data for further studies on immune genes in future.

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