Sequence analysis of the Toll-like receptor 2 gene of old world camels

Shyam S. Dahiya a,*, Govindasamy Nagarajan a, Vijay K. Bharti b, Shelesh K. Swami a, Sharat C. Mehta a, Fateh C. Tuteja a, Shirish D. Narnaware a, Nitin V. Patil a

a National Research Centre on Camel, Post Bag No. 7, Jorbeer, Bikaner 334 001, Rajasthan, India
b DIHAR, DRDO, Ministry of Defence, C/O-56 APO, Leh, 901205, Jammu & Kashmir, India

ABSTRACT

The Toll-like receptor 2 (TLR2) gene of old world camels (Camelus dromedarius and Camelus bactrianus) was cloned and sequenced. The TLR2 gene of the dromedary camel had the highest nucleotide and amino acid identity with pig, i.e., 66.8% and 59.6%, respectively. Similarly, the TLR2 gene of the Bactrian camel also had the highest nucleotide and amino acid identity with pig, i.e., 85.7% and 81.4%, respectively. Dromedary and Bactrian camels shared 77.9% nucleotide and 73.6% amino acid identity with each other. Interestingly, the amidation motif is present in camel (Dromedary and Bactrian) TLR2 only, and the TIR domain is absent in Dromedary camel TLR2. This is the first report of the TLR2 gene sequence of Dromedary and Bactrian camels.

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Introduction

Old world camels (Camelus dromedarius and Camelus bactrianus) have acquired many special abilities and attributes to survive in harsh environments, including cold or hot, arid regions with poor grazing, including deserts or semidesert areas. The camel is less susceptible to many diseases that affect other livestock species, such as brucellosis [1]. Species-specific viral infections, such as camelpox [2] and contagious ecthyma [3], have been reported in Indian Dromedary camels. Camels are susceptible to foot and mouth disease, but no naturally occurring cases seem to occur [4].

Innate immunity is an evolutionarily conserved form of host defense present in invertebrate as well as vertebrate organisms. Activation of innate immunity initiates subsequent adaptive immune responses. The ability to recognize microorganisms depends in part on a family of cell surface transmembrane receptors known as the Toll-like receptors (TLRs). TLRs are among a growing number of receptors that recognize pathogen-associated molecular patterns (PAMPs) as infectious non-self-ligands and, in response, activate an inflammatory cascade that includes recruitment of dendritic cells, the most potent antigen-presenting cells of innate immunity [5]. Although most TLRs appear to function as homodimers, TLR2 forms heterodimers with TLR1 or TLR6, each dimer...
having different ligand specificity, thus increasing its binding repertoire [6]. Individual TLRs trigger specific biological responses. The TLR2–TLR1 heterodimer recognizes triacylated lipopeptides from Gram negative bacteria and mycoplasmas, whereas the TLR2–TLR6 heterodimer recognizes diacylated lipopeptides from Gram positive bacteria and mycoplasmas. TLR2 is also involved in the recognition of viral components such as human cytomegalovirus [7]. Considering the extremely diversified and peculiar features of camels with respect to their tolerance to a variety of climatic conditions and pathogens, the role of innate immunity in camels should not be overlooked. In this study, we sequenced the TLR2 gene of both Dromedary and Bactrian camels to better understand the history of their evolution and to provide a resource for research into the immune system of camels.

**Material and methods**

All animal experiments were performed according to protocols approved by the institutional committee for use and care of animals (Animal ethical clearance No. 354/PCSEA, National Research Centre on Camel, Bikaner, India). Total RNA was extracted from peripheral blood mononuclear cells (PBMCs), and cDNA was prepared using a standard procedure [8] from Dromedary camels maintained at NRCC, Bikaner, India, and Bactrian camels from the State Government Farm, Leh, India. To amplify the TLR2 of Dromedary and Bactrian camel, blood samples were collected by jugular vein puncture, and PBMCs were isolated by density-gradient centrifugation using Histopaque-1077 (Sigma–Aldrich). PBMCs were cultured in RPMI 1640 medium (Gibco BRL) containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 ng/mL streptomycin. Cells (1 × 10^6/mL) were grown in 6-well plates and stimulated with 5 μg/mL lipopolysaccharide (LPS) for 12 h. Total RNA was isolated from LPS-stimulated PBMCs using the RNA isolation kit (Bangalore Genie). An aliquot of total RNA (5 μg) was reverse-transcribed using the Easyscript First Strand cDNA Synthesis Kit (Applied Biological Materials) in a 20 μL volume reaction. TLR2 cDNA was amplified by PCR using primers designed on the basis of TLR2 nucleotide sequence of the pig (Sus scrofa; GenBank GQ304753). Cycling conditions for PCR were 35 cycles at 94 °C for 60 s, 57 °C for 60 s, and 72 °C for 150 s, followed by a final extension at 72 °C for 10 min. Amplified PCR products were separated on 1% agarose gels containing 10 mg/mL ethidium bromide and visualized under ultraviolet light. Purified PCR products were cloned into pGEM-T easy vector (Promega), and the resultant plasmids were transformed into Escherichia coli DH5α. Positive clones were confirmed by colony PCR and restriction analysis with EcoRI and then sequenced in both directions using universal T7 and SP6 primers at Delhi University, India. The sequences were submitted to GenBank, and the assigned accession numbers are JQ979305 (Dromedary camel) and JX453495 (Bactrian camel). The nucleotide and deduced amino acid sequences were analyzed using the BLAST program (NCBI). The resultant nucleotide and amino acid sequences of TLR2 gene from the Dromedary and Bactrian camel were assembled and analyzed with those of fifteen animal species published earlier in the GenBank (Tables 1 and 2) using BioEdit Version 7.0.9. These sequences were compared in Clustal W, and the Phylogenetic tree was constructed in MEGA4 by neighbor-joining method [9]. The functional motifs such as Prokaryotic membrane lipoprotein lipid attachment site, RGD (motif), and amidation of the gene products were predicted by using the computer software GeneRunner version 3.05 (hastings Software Inc. Hastings, NY, USA; http://www.generunner.net). The secondary structure of the TLR2 amino acid sequence is analyzed by protein program of DNASTAR software.

**Results and discussion**

TLR2 cDNA sequences of Dromedary and Bactrian camel were translated into amino acid sequences using EditSeq (DNA STAR). The ORF of Dromedary camel was found to be 1857 bp long encoding for 618 amino acid with molecular weight of 692.72 kDa. The ORF of Bactrian camel has same length of 2358 bp as that of S. scrofa (GQ304753) encoding

| Host             | Country and year of isolation | NCBI accession number | % Identity with Camelus dromedarius |
|------------------|------------------------------|-----------------------|-----------------------------------|
| Camelus dromedarius | India, 2012                  | JQ979305              | –                                 |
| Camelus bactrianus     | India, 2012                  | JX453495              | 77.9                              |
| Antidorcas marsupialis | UK, 2008                   | EU580538              | 66.0                              |
| Bos taurus             | UK, 2006                    | AY634629              | 66.2                              |
| Boselaphus tragocamelus | India, 2011                | DQ286731              | 66.1                              |
| Bubalus bubalis        | India, 2007                 | EU178742              | 66.2                              |
| Canis lupus familiaris | Japan, 2009                | AB189639              | 64.5                              |
| Capra hircus           | India, 2011                 | DQ872435              | 66.3                              |
| Capra ibex             | UK, 2008                    | EU580540              | 66.4                              |
| Cervus nippon          | China, 2011                 | HQ260631              | 66.4                              |
| Equus caballus         | NA                          | NM_001081796          | 66.6                              |
| Giraffa camelopardalis | UK, 2008                   | EU580542              | 66.5                              |
| Gorilla gorilla        | Japan, 2008                 | AB445627              | 64.4                              |
| Homo sapiens           | France, 2009                | DQ012266              | 64.4                              |
| Ovis aries             | UK, 2009                    | AM981300              | 66.2                              |
| Pongo pygmaeus         | Japan, 2008                 | AB445628              | 64.5                              |
| Sus scrofa             | Japan, 2003                 | AB085935              | 66.8                              |

Table 1  Details of TLR-2 gene of Camelus dromedarius nucleotide and amino acid identity with TLR-2 of other species.
785 amino acid but molecular weight of 898.27 kDa as compared to 894.99 kDa of *S. scrofa*.

Among the TLR2 gene of different species, TLR2 gene of the Dromedary camel had the highest nucleotide identity with pig (66.8%), compared to 64.4–66.6% with cattle, sheep, horse, goat, human, and other species of animals (Table 1). At the amino acid level, the predicted TLR2 protein of the Dromedary camel had highest identity with pig (59.6%), compared to 56–59.3% identity with cattle, sheep, horse, goat, human, and other species of animals. The TLR2 gene of the Bactrian camel had the highest nucleotide identity with pig (85.7%) and 82.9–85.6% with cattle, sheep, horse, goat,

### Table 2: Details of TLR-2 gene of *Camelus bactrianus* nucleotide and amino acid identity with TLR-2 of other species.

| Host                  | Country and year of isolation | NCBI accession number | % Identity with *Camelus bactrianus* |
|-----------------------|------------------------------|-----------------------|-------------------------------------|
|                       |                              |                       | Nucleotide                        | Amino acid                      |
| *Camelus bactrianus*  | India, 2012                  | JQ979305              | –                                   | –                                |
| *Camelus dromedarius* | India, 2012                  | JX453495              | 77.9                               | 73.6                             |
| *Antidorcas marsupialis* | UK, 2008                  | EU580538              | 85.1                               | 79.8                             |
| *Bos taurus*          | UK, 2006                     | AY634629              | 85.2                               | 80.7                             |
| *Boselaphus tragocamelus* | India, 2011               | DQ286731              | 85.3                               | 79.8                             |
| *Bubalus bubalis*     | India, 2007                  | EU178742              | 85.4                               | 80.6                             |
| *Canis lupus familiaris* | Japan, 2009             | AB189639              | 83.1                               | 78.0                             |
| *Capra hircus*        | India, 2011                  | DQ872435              | 85.1                               | 80.1                             |
| *Capra ibex*          | UK, 2008                     | EU580540              | 85.3                               | 81.0                             |
| *Cervus nippon*       | China, 2011                  | HQ260631              | 85.4                               | 80.6                             |
| *Equus caballus*      | NA                           | NM_001081796          | 85.6                               | 81.2                             |
| *Giraffa camelopardalis* | UK, 2008               | EU580542              | 85.4                               | 80.5                             |
| *Gorilla gorilla*     | Japan, 2008                  | AB445627              | 82.9                               | 77.4                             |
| *Homo sapiens*        | France, 2009                 | DQ012266              | 82.9                               | 77.4                             |
| *Ovis aries*          | UK, 2009                     | AM981300              | 84.9                               | 80.2                             |
| *Pongo pygmaeus*      | Japan, 2008                  | AB445628              | 83.1                               | 77.5                             |
| *Sus scrofa*          | Japan, 2003                  | AB085935              | 85.7                               | 81.4                             |

![Fig. 1](image)  
**Fig. 1** Phylogenetic tree based on amino acid sequences of TLR2 gene of *Camelus dromedarius*. The tree was constructed by neighbor-joining method using Mega 4 (Molecular Evolutionary Genetics Analysis) software with bootstrap values calculated for 1000 replicates. Horizontal distances are proportional to the genetic distances. Vertical distances are arbitrary. The numbers at each branch represent bootstrap values (1000 replicates). The scale bar at the bottom measures the nucleotide distance.
human, and other species of animals (Table 2). At the amino acid level, the predicted TLR2 protein of the Bactrian camel had the highest identity with pigs (81.4%), compared to 77.4–81.2% with cattle, sheep, horse, goat, human, and other species of animals. Dromedary and Bactrian camels shared 77.9% nucleotide and 73.6% amino acid identity with each other. Homologues of human TLRs 1–10 are present in both cattle and sheep, with >95% and 83–90% nucleotide sequence identity to the corresponding human reference sequences, respectively, while the degree of conservation of amino acid sequences between homologous ruminant and human TLRs is 84–97% [10]. Phylogenetic analysis of 17 TLR2 nucleotide sequences from different species using the MEGA4 program showed that the TLR2 sequence of Dromedary and Bactrian camels cluster together (Fig. 1).

The ORF of both, i.e., Dromedary and Bactrian camel TLR2 has one amidation motif at position 572 (marked with star symbol in Fig. 2). One RGD motif, i.e., cell attachment

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**Fig. 2** Alignment of amino acid sequences of TLR2 encoding gene of Dromedary and Bactrian camel with TLR2 of other species, using the software BioEdit Version 7.0.9. Star indicates the position of amidation motif in Dromedary and Bactrian camel TLR2. Arrow denotes the RGD motifs in Bactrian and pig TLR2 and triangle denotes the position of Prokaryotic membrane lipoprotein lipid attachment site in Dromedary camel TLR2. Shaded areas indicate the conserved amino acids in the protein described.
sequence is present at position 637 and 293 in Bactrian and pig TLR2, respectively (marked with arrow symbol in Fig. 2). A Prokaryotic membrane lipoprotein lipid attachment site is present only in Dromedary camel at position 606 (marked with a triangle symbol at position 678 in Fig. 2), which is absent in all other 16 TLR2 sequences considered for analysis in the present study. It is interesting to observe that TLR2 of Dromedary camel lacks a TIR domain and a transmembrane region whereas both of these are present in Bactrian camel. These motifs may be responsible for imparting the specific biological activity to the TLR2 receptors, which further needs to be investigated experimentally so as to provide insight into their role in resistance to bacterial and viral pathogens.

The secondary structure of the TLR2 amino acid sequence is analyzed by protean program of DNASTAR software. Secondary structure analysis of TLR protein sequence of C. dromedarius showed higher propensity of beta sheet as compare to alpha helix. On the other hand, TLR protein sequence of C. bactrianus showed higher propensity of alpha helix as
compare to beta sheet. Both the TLR showed higher antigenicity index toward the C terminus.

Conclusions

This is the first report of the TLR2 gene sequence in Dromedary and Bactrian camels, and this information may be useful for studies of evolutionary lineages, phylogenetic analysis, and immune functions associated with bacterial infection of camels.

Conflict of interest

The authors have declared no conflict of interest.

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**Fig 2. (continued)**

| Species                  | Sequence                                |
|--------------------------|-----------------------------------------|
| Canis lupus familiaris   | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Gorilla gorilla          | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Pongo pygmaeus           | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Ovis aries               | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Bos taurus               | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Homo sapiens             | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Boselaphus tragocamelus  | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Bubalus bubalis          | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Capra hircus             | ISELYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Antidorcas marsupialis   | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Capra ibex               | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Giraffa camelopardalis   | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Sus scrofa               | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Cervus nippon            | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Camelus bactrianus       | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Camelus dromedarius      | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |
| Equus caballus           | KSLYLDLSENLMVDEHKNSSACFASVQGLLVKRNH   |

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