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MOLECULAR CHARACTERISATION OF DIACYLGLYCEROL O-ACYLTRANSFERASE (DGAT1) GENE IN DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

Present study was undertaken to characterise Diacylglycerol O-Acyltransferase (DGAT1) gene of dromedary camels (*Camelus dromedarius*). Molecular characterisation of 773 bp long DGAT1 gene fragment was performed in Indian dromedary camel through PCR amplification, sequencing and bioinformatics analysis. The characterised DGAT1 gene fragment covered partial exon-6, exon-7, 8, 9 and partial exon-10 and complete intron-6, 7, 8, 9 regions. A single nucleotide variation was seen at position 463 C>T of exon-8 in both Bikaneri and Kachchhi breeds. Animals with single and double peaks in the sequence chromatograms were observed at this particular locus. Accordingly, two alleles (C, T) and only two genotypes (CC and CT) were identified in the Bikaneri and Kachchhi camel breeds. Bikaneri and Kachchhi camel (*Camelus dromedarius*) sequence showed highest similarity (99.71%) with predicted dromedary camel, Ferus (wild) camel, Bactrian camel and Vicugna DGAT1 sequences. With other species like cattle, buffalo, pig, sheep, goat, yak, dog, cat, killer whale, naked mole rat and human DGAT1 sequence similarity ranging from 73.55 % to 84.93 % was observed. Pig was closest species (84.93%), whereas American Shorthair breed of cat (78.08%) was least similar to dromedary camel. Similar relationship was observed between species on the phylogenetic analysis of DGAT1 gene nucleotide sequences.

Key words: Camel, DGAT1 gene, dromedary, phylogeny

The vital role of DGAT1 in fat metabolism makes them a best choice as a candidate marker in animal production (Khan *et al,* 2021). DGAT1 was documented to have a significant influence on milk production in cattle in Germany (Molee *et al,* 2012). Diacylglycerol O-acyltransferase 1 (DGAT1) was identified as one underlying QTL for milk production traits located on the centromeric region of the bovine chromosome 14 having 17 exons with 14,117 base pair (bp) (Grisart *et al,* 2002 and Winter *et al,* 2002) and was located on chromosome 15 has a size of 10,733 bp distributed in 19 exons in buffaloes (Amaral *et al,* 2008).

The marker genes for camel milk traits can give the pathway to improving camel milk yield and quality which can result in increased economic importance of camel. The polymorphism in candidate genes like CSN1S1, CSN1S2, CSN2, CSN3, ACACA, DGAT1, DGAT2,ME1, SCD, LPL, LIPE, BTN1A, MFGE, GH, PRLR, PITX2, POUF1, and STAT5 has been linked with milk yield and composition traits (Tesema and Alemayehu, 2018). So far only some information on polymorphism in Casein gene (Gahlot *et al,* 2019; Jadhav *et al,* 2019; Jadhav *et al,* 2020), Growth hormone gene (Prakash *et al,* 2021; Jyotsana *et al,* 2021) and Leptin gene (Choudhary *et al,* 2022) have been reported in the case of Indian dromedary camel. DGAT1 gene have one of most important QTL associated with variation in milk composition and parameters (Fernir *et al,* 2002; Kuhn *et al,*2004). Structure and role of DGAT1 gene has been widely studied in cattle, buffalo and to some extent in sheep, goat. Allelic variants of DGAT1 are associated with Fat %, Protein %, Lactose %, and SNF % and milk yield (Thaller *et al,* 2003; Hanusova *et al,* 2014; Faraj *et al,* 2020).

Samuel *et al* (2022) included fifteen haplotypes/ sequences of milk producing farm animals from Genbank including Bos taurus (AJ318490, EU077528, MF069174 and MF445056), *Bubalus bubalis* (MZ230553, MZ230553, MF069172 and KX965992), *Camelus dromedarius* (MF069170 and MF069171), *Capra hircus* (LT221856 and FJ415876) and *Ovis aries* (KJ918741, FJ415875 and EU178818) from Germany, Turkey, India, Iran and Benin in the analysis.

The whole genome Illumina assemblies or shotgun sequence (CamDro3) of chromosome 25 on

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North African dromedary camels reported camel DGAT1 gene extends from 41215260 to 41224586 bases (GenBank: NC_044535.1) with a size of 9327 bp (Elbers *et al,* 2019). It splits into 16 exons and 15 introns (Gen Bank: KAB1258102.1). The molecular structure of DGAT1 gene in Indian dromedary and bactrian camels are not available. Hence, the present investigation was undertaken for molecular characterisation of DGAT1 gene in Indian dromedary camels.

Materials and Methods

Blood samples were randomly collected from 10 camels each from Bikaneri and Kachchhi breeds maintained at ICAR-National Research Centre on Camel (NRCC) farm at Bikaner, Rajasthan (India). The DNA was extracted from blood cells using standard phenol-chloroform extraction protocol (Sambrook *et al,* 2001). PCR amplification of 773 bp DGAT1 gene fragment was performed utilising primers designed with the help of Primer-BLAST system of NCBI (Stephen *et al,* 1997) and were synthesised from Eurofins genomics. Primer sequence (5'-3') in Forward direction was CCTTCCTGCTTGAGTCCAT, whereas reverse primer was ACTTGGAGCTGGGTAAGG. The gradient PCR programme was used to find out the appropriate annealing temperature. The PCR reaction was carried out with Green master mix-13 µl (Promega, USA), 1µl of each primer with concentration of 10 pM, 1µl of 80-100 ng camel genomic DNA and nuclease free water (Promega, USA) to make total volume up to 25µl. Amplification was performed in thermal cycler (Applied biosystem Veriti™ Thermal cycler) programmed for initial denaturation at 95°C for 5 min, followed by 34 cycles of denaturation at 94°C for 60 s, annealing at variable temperatures for 45 s, extension at 72°C for 45 s and final extension at 72°C for 10 min. The PCR products were checked for amplification by electrophoresis on 1.0% agarose gel (Himedia) in parallel with 1kb plus DNA marker (Thermo-scientific). Standardised temperature was further used for the amplification of all the samples. This bidirectional sequencing using forward and reverse primers was performed using Sanger Dideoxy Chain termination method (GeneOmBio Technologies Pvt. Ltd.). The forward, reverse sequences obtained for each animal were edited using Codon Code Aligner software (USA) and different sequences patterns were generated. The pair wise and multiple alignment of the different sequence pattern were analysed to find out the differences and relationship between Indian camels DGAT1 gene sequences. Obtained sequences were compared with camelid and other domesticated species DGAT1 gene sequences available in National Centre for Biotechnology Information (NCBI) database using BLAST software program (http://www. ncbi. nlm.nih.gov/) to study the sequence variation and relationship. The estimation of evolutionary relationship between different species was inferred by neighbour joining method by using the Blast Tree View system of online BLAST® software of NCBI and phylogenetic tree was also studied.

Results and Discussion

The annealing temperature of 57^{o} C was found optimal for amplification of the target DGAT1 gene fragment. Single clear bands were observed, when the PCR products were checked for amplification by electrophoresis on 1.0 % agarose gel in parallel with 1 bp plus DNA marker (Fig 1).

After bidirectional sequencing of PCR products, visualisation of sequence chromatograms and editing of sequence reads using Codon Code aligner software, 773 base pair DGAT1 gene fragment and its genetic variants were identified. The amplified DGAT1 gene fragment covered partial exon-6, complete intron-6, 7, 8, 9, complete exon-7, 8, 9 and partial exon-10.

Population genetic differentiations based on the DGAT1 genes of the breeds had been evaluated by Ne's genetic distance (GST) by DnaSP software (Librado *et al,* 2009).

Nucleotides were conserved at all the positions in 773 bp long DGAT1 gene fragment sequence, except 463rd position which was part of exon-8. At position 463 the sequence chromatogram of some animals showed one peak and some had two peaks due to presence of different allele at the same locus. This variation was due to change in nucleotide sequence 463 C>T in Bikaneri and Kachchhi breed. The nucleotide variation caused change in last codon of 8th exon from CGC to CGT. However, it was a synonymous mutation as both codons code same amino acid (Arginine). The nucleotide change resulted in 2 allelic (C, T) and 3 genotypic variations (CC, CT, and TT) in Indian dromedary camels (Fig 2).

The sequence variation and identity percentage were determined on the basis of pairwise nucleotide BLAST of Indian dromedary DGAT1 gene (C allele) with Ferus (wild), Bactrian, dromedary camel, vicugna, and other species like cattle, buffalo, pig, sheep, goat, yak, dog, cat, killer whale, naked mole rat and human. Dromedary DGAT1 gene sequences with 773 fragment size showed 99.17% identity with predicted DGAT1 cDNA sequences of *Camelus ferus*,

dromedary and Bactrian camel. Per cent identity of Indian dromedary with other species varied between 73.55 % to 84.93 % (Table 1). The differences could be due to transition, transversion, insertion and deletion type of mutations during the course of evolution. This DGAT1 gene is present on different chromosomes between DGAT1 genes (Fig 3) of different species as observed on the basis of percentage identity of the nucleotide sequences.

Multiple Sequence alignment was prepared between DGAT1 gene and DNA, cDNA sequences from different species with NCBI MSA programme

Table 1. Percentage identity between DGAT1 gene sequence of Indian dromedary camel with DGAT1 gene sequences of another species.

Accession Id.	Organism and type of Reference Sequence	Query Cover	% Identity	Acc. Len
XM_032467564.1	PREDICTED: *Camelus ferus* DGAT1, mRNA	43%	99.17%	1708
XM_006211243.3	PREDICTED: *Vicugna pacos* DGAT1, mRNA	42%	99.17%	2205
XM_031439877.1	PREDICTED: *Camelus dromedarius* DGAT1, variant X1	44%	99.17%	1559
XM_010961176.1	PREDICTED: *Camelus bactrianus* DGAT1, partial mRNA	43%	99.17%	1624
AY116586.1	Sus scrofa DGAT1 gene, complete cds	89%	84.93%	9303
OW443377.1	*Orcinus orca* genome assembly, chromo:17	99%	84.81%	87542868
AY999090.1	*Bubalus bubalis* DGAT1 gene, complete cds	91%	80.06%	12372
CP027082.1	*Bos mutus* isolate yakQH1 chromosome 14	77%	79.83%	81354091
EU178818.1	*Ovis aries* DGAT1 gene, complete cds	87%	79.66%	8676
LR962869.1	*Bos taurus* genome assembly, chromo: 14	83%	79.46%	82271483
EF636701.1	*Bos indicus* breed Butana DGAT gene, partial cds	83%	79.46%	8107
LT221856.1	*Capra hircus* dgat1 gene	87%	79.34%	37251
CP050604.1	*Canis lupus* familiaris breed Labrador retriever chromosome 13a	86%	79.23%	63905973
AP023169.1	*Felis catus* Senzu DNA, chromo: F2, American Shorthair breed	100%	78.08%	86116642
OX090950.1	*Hetero cephalus* glaber genome assembly, chromosome: 10	82%	73.95%	94825129
NG_034192.1	*Homo sapiens* DGAT1, RefSeq Gene chromosome 8	90%	73.55%	19337

in different species, as it is present on chromosome number 25 in members of Camelidae like wild camel, vicugna, dromedary and double humped Bactrian camel. Its location in human, cattle, buffalo, sheep, pig, dog, cat and whale is on chromosome number 8, 14, 15, 9, 4, 13, 2 and 17, respectively. Its nature as transposon or jumping gene (Pray, 2008) can also be investigated. The evolutionary relationship inferred using Neighbour joining method of phylogeny tree construction showed similar relationship pattern

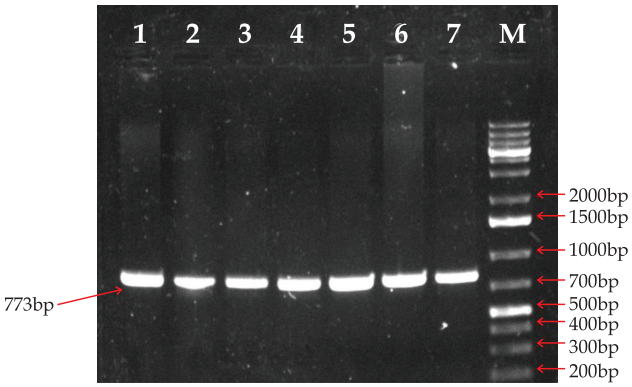

Fig 1. Diacylglycerol O-Acyltransferase (DGAT1) gene resolved on 1.0% agarose gel Lane-Marker 1k bp plus DNA ladder (M), Lane-1 to 7 are DGAT1 gene product.

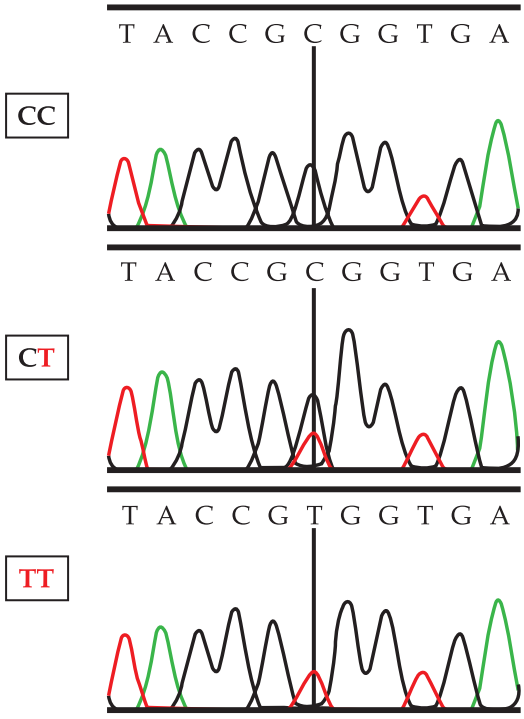

Fig 2. Sequence chromatogram depicting nucleotide change at position 463 in DGAT1 gene sequence of Indian dromedary camel. Different colour and type of peak at position 463 show genotypes pattern

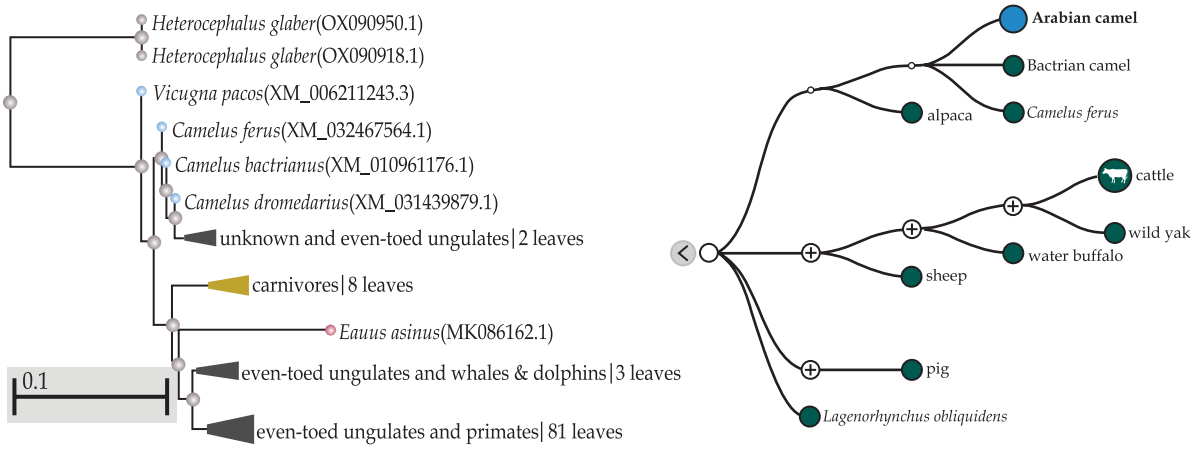

Fig 3. Phylogeny tree of DGAT1 gene based upon Neighbour joining method.

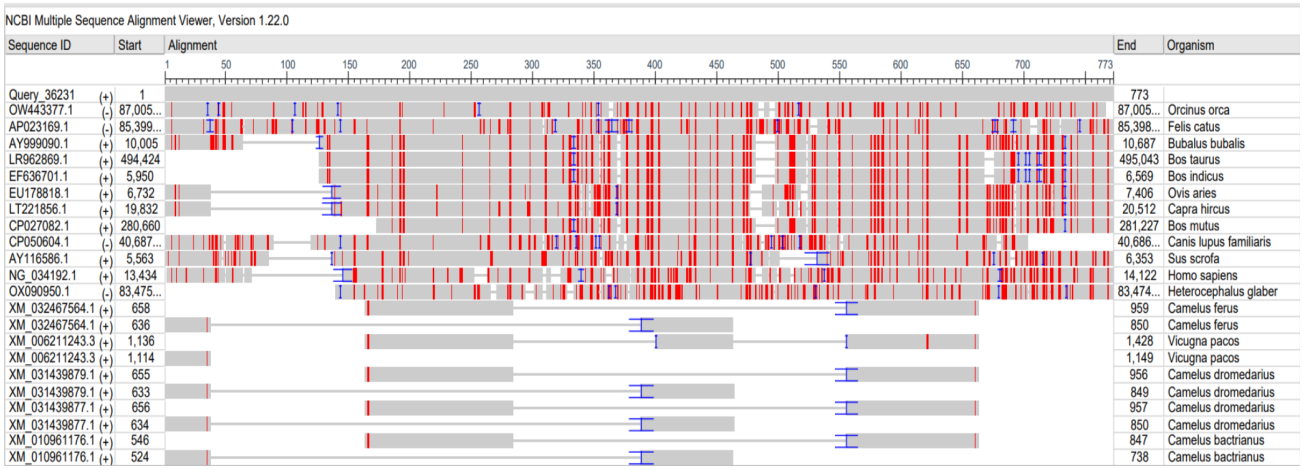

Fig 4. Multiple Sequence alignment between DGAT1 gene and cDNA/DNA sequences from different species. (At extreme left of Fig Sequence ID is denoted as accession number, at extreme right of Fig zoological names of species are mentioned).

(Ver: 1.22.0), which show all possible genetic similarities and differences between targeted 15 species (Fig 4).

A study conducted by Samuel *et al* (2022) found that the DGAT1 gene may provide baseline information for in-depth understanding, exploitation of milk linked gene variation and could be used as a marker in selection programmes to enhance the production potential and genetic gain in Ethiopian cattle populations.

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