MOLECULAR CHARACTERISATION OF KERATIN-ASSOCIATED PROTEIN-7 (*KRTAP7*) GENE FOR HAIR QUALITY IN INDIAN DROMEDARY CAMEL

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ABSTRACT

The keratin-associated proteins (*KRTAPs*) play important role in describing the physical and mechanical properties of the fibre *viz*. fibre thickness and curliness to the hair fibres. In this report, the molecular and functional characterisation of the *KRTAP7* gene in four Indian camel breeds i.e. Bikaneri, Jaisalmeri, Kachchhi and Mewari is described based on a comprehensive analysis of the nucleotide and amino acid sequences. The blood sample was collected from representative animals differing in fibre quality. The *KRTAP7* gene was amplified, sequenced and analysed for genetic characterisation. The coding sequence of the *KRTAP7* gene was of 264bp. The *KRTAP7* gene sequences from all four breeds of camel were identical. No SNPs were noticed in coding (CDS) and non-coding (5' UTR and 3' UTR) regions of *KRTAP7* gene. The complete coding sequence (CDS) of the *KRTAP7* gene translated to 87 amino acids (aa) long *KRTAP7* protein. The phylogeny analysis of nucleotide and protein sequence of Indian dromedary *KRTAP7* gene revealed closet relationship with *Camelidae* family. This study provides information about the *KRTAP7* gene in Indian dromedary camel.

Key words: Camel, dromedary, hair fibre, keratin-associated protein-7 (KRTAP7) phylogeny

Coat colour and fleece type traits have pleiotropic effect at genetic level and comes under qualitative traits as their expression is dependent on one or several genes with little or no environmental effects. On the other hand, the amount of fibre produced or fineness (diameter) have polygenic effect and come under quantitative traits as determined by several genes with minor effects per gene while major environment effect (Mackay, 2003).

Calderon *et al* (2021) identified set of SNPs located at or nearby candidate genes (*KRT, KRTAP, ASIP, MC1R, TYRP1*) for fibre quality and colour in *Huacaya alpacas* by genotyping by sequencing (GBS). Some keratin (*KRT*) and keratin-associated protein (*KRTAP*) genes are important candidate genes for fleece and fibre quality (Allain and Renieri, 2010). The process of keratinisation is most important among skin adaptations which helps in positive selection mechanism by interaction of fibrous (keratin) and matrix proteins (*KRTAPs*) (Alibardi *et al*, 2009; Rogers *et al*, 2007). Fibre is produced by secondary hair follicles which are composed of hair-keratins and

keratin-associated proteins (KAPs). These multigene subfamilies encoded KAP proteins have specific in high or ultra-high sulphur (contributed by cysteine) content (HS) and a high glycine-tyrosine (HGT) (Wu et al, 2008). Such proteins also define the particular structural and chemical characteristics of the different types of hair, fur, wool and quills in various animals (Fujimoto et al, 2014). The discovery and analysis for variation in the quality of hair/fibre revealed over 100 and 88 keratin-associated proteins (KRTAPs) which have been identified in various mammalian species and human, respectively (Khan et al, 2014; Rogers et al, 2008). KRTAPs play an important role in describing the physical and mechanical properties of the fibre viz. fibre thickness and curliness to the hair fibres by acting as a matrix for cross linking the hair-keratins (Gong et al, 2016; Li et al, 2019). The different evolution rate is responsible for a high degree of diversification and homogenisation in KRTAP7 gene family with more than 30 subfamilies. Our study considered molecular characterisation of the KRTAP7 gene for hair quality traits in Indian dromedary camel.

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The pseudogenisation rate in one or more proteins from the repertoire of the KRTAP genes family are responsible for the diversification patterns in hair fibre in different species such as in sloth (175 KRTAP genes with 141 being intact for long and coarse hair) and dolphin (35 KRTAPs). The modification of hair structure and function due to changes in the KRTAP gene repertoire are responsible for surface adaptation in different species such as alpaca (fibre), armadillo (modified scales), hedgehog (spines) and dolphin (mostly hairless and aquatic). However, the reports on molecular characterisation of the KRTAP7 gene with respect to the Indian camel is scanty. The present study was therefore, designed with the objective of characterising the KRTAP7 gene and translated protein in Indian dromedary camel.

Materials and Methods

Sampling structure

The sampling was done under permission of Institutional Ethics Committee. The blood samples were collected from the jugular veins in BD Vacutainer® blood collection tubes from 35 camels of 4 breeds namely Bikaneri (10), Jaisalmeri (9), Kachchhi (6) and Mewari (10). The samples were collected from the institutional camel herd of ICAR-National Research Centre on Camel (NRCC) Bikaner, India. The animals were selected on the basis of hair quality attributes such as fibre diameter and medullation percent. The fibre diameter (μ) from sampled animals was ranging between 21.10-36.56 for Bikaneri, 24.44-73.61 for Jaisalmeri, 26.54-60.83 for Kachchhi and 36.98-62.19 for Mewari breed. The medullation per cent was ranging between 13.33-81.33 for Bikaneri, 32.33-97.00 for Jaisalmeri, 33.00-93.67 for Kachchhi and 39.00-94.00 for Mewari breed in sampled animals.

Molecular characterisation of the KRTAP7 gene

The total DNA was isolated from blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Aarhus, Denmark) as per manufacturer's guidelines. The *KRTAP7* gene fragment was amplified from isolated genomic DNA samples using custom designed primers (Forward: 5'AGGTATCACCCTATCCTGGTGT3'; Reverse: 5'AGTCTGTGGGGCTCCT TTGTATG3') using reference genome sequence locus [NC_044511]. The PCR reaction mix (25 μ L) for amplification of *KRTAP7* gene fragment was prepared by adding 1 μ L of each primer (10 pmol), 2 μ L of genomic DNA, 13 μ L of Taq PCR Master Mix (Qiagen, USA) and 8 μ L of Nuclease free water. The PCR reaction was performed in Veriti® thermocycler (Applied Biosystems, USA) using the time-temperature combination: an initial denaturation at 95° C for 5 min followed by 34 cycles of denaturation (94°C for 1 min), annealing (59.5°C for 45 sec) and extension (72 °C for 1 min) followed by a single cycle of final extension (72°C for 10 min). A single band of 995 bp was observed when the PCR products were checked for amplification by electrophoresis on 1.0% agarose gel (Himedia), in parallel with 100-1500bp NEX-GEN DNA ladder (Puregene, Genetix Biotech Asia Pvt. Ltd.) (Fig 1). The PCR products were purified using Wizard® SV Gel and PCR Clean-Up System kit (Promega Corporation, USA). The bidirectional sequencing of the purified KRTAP7 amplicons were performed by Sanger's dideoxy chain termination method (Eurofin Genomics, USA). The forward and reverse sequences were manually curated and analysed using Codon Code Aligner Software (Codon Code Corporation, USA). The KRTAP7 sequences generated for Indian dromedary camel breeds were compared with the reference genome sequence locus [NC_044511] using BLAST software program (http://www.ncbi.nlm.nih.gov/). The pair wise and multiple sequence alignments of identified Indian camel KRTAP7 gene sequence were done to study the variation and identity with other reported camelid and the sequences of domesticated species using Clustal2.1(https://www.ebi.ac.uk/Tools/ msa/clustalo/n) and dismat-EMBOSS (https:// www. ebi.ac.uk/Tools/emboss/) software package. The estimation of evolutionary relationship and phylogenetic tree construction (Table 1) between nucleotide sequences and the deduced amino acid sequences of different species and Indian dromedary camel were inferred by Maximum Likelihood bootstrapped method using Molecular Evolutionary Genetics Analysis software (MEGA 11.0) (Tamura et al, 2021).

Results and Discussion

Molecular characterisation of KRTAP7 gene

The amplification and sequencing of *KRTAP7* gene was done in Indian dromedary camel breeds. The 995 base pairs long fragment of *KRTAP7* gene was amplified (Fig 1). The sequencing of the PCR products from 35 animals resulted in retrieval of 915 bp long sequence after alignment and editing of the forward and reverse sequences. The 915 bp long sequence covered 264 bp long coding region and 518 bp long non-coding 5' UTR region and 133 bp long 3' UTR region. The 264 bp long coding ORF domain

of *KRTAP7* gene translated into 87 amino acids (aa) length protein sequence in Indian dromedary camel breeds.

No SNPs were noticed in coding (CDS) and non-coding (5' UTR and 3' UTR) regions of KRTAP7 gene. In resemblance to our study, no polymorphism in the CDS region of KRTAP7 gene was observed in bovine species such as Chinese yak breeds and Indonesian zebu cattle (Arlud et al, 2017). However, in contrast to present study the identified novel SNPs at different positions of KRTAP7-1 were associated with the mechanical strength and shape of hair fibre in llama (c.99C>T, c.185G>A, c.198T>C and c.237C>G) (Daverio et al, 2019) and sheep (c.93C>A and c.173G>A) (Liu et al, 2014; Gong et al, 2012). The genetic variants with AA (n = 169) and AB (n = 51) genotypes were associated with wool production traits such as mean yield (79.9 \pm 2.72% and 81.9 \pm 3.37%) and mean staple length (MSL: 47.3 ± 0.57 mm and 50.9 ± 0.65 mm), respectively in Pakistani sheep breeds (Ullah et al, 2020). The hair characteristics are controlled by vast repertoire of keratin gene family such as 80 KRTAPs (25 families) in humans (Gong et al, 2012), 29 KRTAPs (13 families) in sheep (Li et al, 2017), 12 KRTAPs (9 families) in goats (Wang et al, 2017), 5 keratin (KRT) gene and 4 candidate gene as makers (BMP4, COL1A2, GLI1, SFRP4) in alpaca (Mendoza et al, 2020). Monomorphic nature of *KRTAP7* gene in yak and Indian dromedary camel indicates very limited variations, its unique intrinsic structural property (e.g., > 21% high glycine content) and vital functional role in strength and shape of hair fibre. Conclusively, the definite role of KRTAP7

gene in wool traits *viz.* yield and structural variation was indicated in some mammalian species but not in Indian dromedary.

The CDS region and translated protein sequence for KRTAP7 were subjected for comparative per cent identity analysis with different mammalian species (Table 2 and 3). The local alignment (NCBI-BLASTN and NCBI-BLASTP), identity matrix and distance matrix of targeted KRTAP7 proteins indicated 100% identical sequence with the keratin associated protein7-1 gene of Camelus dromedarius (reference sequence). The lowest divergence for gene (72.47) and protein (4.60) was noticed with Camelus bactrianus and Vicugna pacos, respectively. However, Camelus bactrianus and Camelus ferus also showed 100% identity with each other among all compared sequences. Highest divergence for targeted protein was shown with Sus scrofa (79.90) and Capra hircus (47.06) while lowest identity was with Mus musculus (74.42). The, overall highest divergence for KRTAP7 proteins (49.41) was noticed between Mus musculus and Capra hircus (Table 3).

Phylogenetic analysis

Phylogram revealed the dissemblance of *Sus scrofa*, *Capra hircus*, *Equus caballus*, *Mus musculus*, *Homo sapiens*, *Vicugna pacos*, *Bubalus bubalis* and from that of *Camelus dromedarius* during evolution. Phylogenetic relationship among considered nucleotide and amino acid sequences was established to infer the evolutionary history using the Maximum Parsimony method with 500 bootstrapped replicates (Fig 2 and 3). Multiple protein alignments for considered species was shown accordingly in Fig 4.

Accession no. Gene CDS S. No. Chr no. Protein (AA) Species Reference acc no. (Protein) (bp) (bp) 1 Camelus dromedarius NC_044511.1 1 KAB1284088.1 732 264 87 2 Camelus bactrianus NW_011514130.1 Un XP_010954900.1 730 264 87 3 Camelus ferus NC_045696.1 1 EQB78523.1 729 264 87 4 Vicugna pacos NW 021964153.1 1 XP_006216075.1 734 264 87 5 Homo sapiens NC_000021.9 21q22.11 NP_853637.2 721 264 87 16; 16 C3.3 NP_082047.1 87 6 Mus musculus NC_000082.7 621 264 7 Equus caballus NC_009169 XP_014591977.1 739 87 26 264 8 Sus scrofa NC_010455.5 13 XP_003358967.2 597 258 85 9 Bubalus bubalis NC_059157.1 1 XP_006053907.3 740 264 87 10 Bos taurus KJ551549.1 1 AHZ89844.1 285 87 264 11 Capra hircus NC_030808 1 QES86378.1 707 258 85 NC_056054 1 QPP12018.1 676 258 85 12 Ovis aries

Table 1. Details of sequences subjected for comparison for KRTAP7 gene.

acc. no.= Accession number; Chr no.= Chromosome number; Un= Unknown; bp=base pairs; CDS= Coding sequence; AA=Amino acids

S no	Accession no	1	2	3	4	5	6	7	8	9	10	11	12	13
5.110.	7Accession no.	1	-	5	T	5	<u> </u>	,	0	,	10	- 11	12	15
1	Camelus		36.90	97.39	100.00	99.18	99.18	82.35	82.06	83.49	82.04	89.77	79.40	38.41
2	NC_000082.7	74.56		63.13	63.58	63.74	63.74	61.52	66.22	66.16	63.99	76.89	64.57	64.14
3	NW_021964153.1	76.70	76.49		97.26	96.84	96.98	80.70	78.17	80.55	78.88	89.39	77.12	76.28
4	NC_044511.1	76.50	76.01	76.09		99.18	99.18	80.34	78.87	81.32	78.98	89.77	77.41	76.43
5	NW_011514130.1	72.47	71.18	75.48	76.30		100.00	80.34	78.59	80.85	78.79	89.77	77.12	76.43
6	NC_045696.1	75.03	76.81	71.74	72.98	73.94		80.34	78.59	80.83	78.90	89.77	77.12	76.43
7	NC_010455.5	79.90	76.55	72.70	74.37	78.39	74.04		79.05	83.25	83.53	91.09	81.59	80.32
8	NC_000021.9	75.59	43.96	76.42	74.48	69.21	75.45	75.88		82.73	76.40	89.02	75.15	73.95
9	NC_009169.3	75.51	80.03	43.19	78.14	75.89	73.25	72.36	74.48		79.89	90.53	78.77	78.16
10	NC_059157.1	73.92	74.88	72.89	70.08	72.33	75.45	72.19	71.71	62.92		98.11	94.89	93.48
11	KJ551549.1	76.89	73.11	78.79	71.59	73.86	64.02	78.79	76.89	78.41	76.52		96.12	96.12
12	NC_030808.1	77.51	74.24	72.14	78.64	74.82	66.20	71.52	75.53	72.28	76.66	74.62		98.08
13	NC_056054.1	73.52	71.50	76.48	73.22	66.57	75.15	76.38	73.08	72.78	72.78	74.24	74.41	

Table 2. Per cent Identity Matrix of KRTAP7 gene between Indian dromedary camel and different species subjected for comparison.

Above diagonal: Per cent identity; below diagonal: Divergence

NC_000082.7: Mus musculus; NW_021964153.1: Vicugna pacos; NC_044511.1: Camelus dromedarius; NW_011514130.1: Camelus bactrianus; NC_045696.1: Camelus ferus; NC_010455.5: Sus scrofa; NC_000021.9: Homo sapiens; NC_009169.3: Equus caballus; NC_059157.1: Bubalus bubalis; KJ551549.1: Bos taurus; NC_030808.1: Capra hircus; NC_056054.1: Ovis aries.

Table 3. Per cent Identity Matrix of KRTAP7 protein between Indian dromedary camel and different species subjected for comparison.

S.no.	Accession no.	1	2	3	4	5	6	7	8	9	10	11	12	13
1	NP_082047.1		77.38	76.74	78.57	80.23	79.07	78.57	80.23	72.09	74.42	74.42	75.58	75.58
2	XP_003358967.2	49.41		85.88	86.75	87.06	87.06	86.75	85.88	82.35	84.71	84.71	84.71	84.71
3	XP_014591977.1	27.59	41.18		89.41	89.66	89.66	89.41	89.66	87.36	89.66	89.66	89.66	89.66
4	QES86378.1	49.41	23.53	48.24		96.47	96.47	97.65	88.24	85.88	88.24	88.24	88.24	88.24
5	AHZ89844.1	25.29	38.82	10.34	43.53		97.70	96.47	88.51	86.21	88.51	88.51	88.51	88.51
6	XP_006053907.3	26.44	40.00	10.34	44.71	2.30		96.47	88.51	86.21	88.51	88.51	88.51	88.51
7	QPP12018.1	48.24	23.53	47.06	2.35	42.35	43.53		88.24	85.88	88.24	88.24	88.24	88.24
8	NP_853637.2	24.14	42.35	10.34	47.06	11.49	11.49	45.88		86.21	88.51	88.51	88.51	88.51
9	XP_006216075.1	32.18	42.35	12.64	48.24	13.79	13.79	47.06	13.79		95.40	95.40	93.10	93.10
10	Camelus	29.89	41.18	10.34	47.06	11.49	11.49	45.88	11.49	4.60		100.00	97.70	97.70
11	KAB1284088.1	29.89	41.18	10.34	47.06	11.49	11.49	45.88	11.49	4.60	0.00		97.70	97.70
12	XP_010954900.1	28.74	41.18	10.34	45.88	11.49	11.49	44.71	11.49	6.90	2.30	2.30		100.00
13	EOB78523 1	28 74	41 18	10.34	45.88	11 49	11 49	44 71	11 49	6.90	2 30	2 30	0.00	

Above diagonal: Percent identity; below diagonal: Divergence

NP_082047.1: Mus musculus; XP_003358967.2: Sus scrofa; XP_014591977.1: Equus caballus; QES86378.1: Capra hircus; AHZ89844.1: Bos taurus; XP_006053907.3: Bubalus bubalis; QPP12018.1: Ovis aries; NP_853637.2: Homo sapiens; XP_006216075.1: Vicugna pacos; KAB1284088.1: Camelus dromedarius; XP_010954900.1: Camelus bactrianus; EQB78523.1: Camelus ferus.

The phylogeny tree based on nucleotide and amino acid sequence for the KRTAP7 gene depicted different evolutionary patterns among species considered for evolutionary relationship analysis. Both the phylogeny trees depicted similar relationship patterns between Camelus ferus and Camelus dromedarius in comparison to other Camelidae species. The highest amino acid sequence homology was noticed among the Camelidae family followed by the Homo sapiens, Mus musculus, Sus scrofa, Equus caballus and other species. The nucleotide sequences of Capra hircus were farthest related compared to other bovine (cattle, buffalo) and ovine species. The multiple protein sequence analysis revealed the highest amino acid similarity between camelids and the highest amino acid differences with Mus musculus (Fig 4)

This maiden report describes the molecular characterisation of the *KRTAP7* gene and protein in four Indian dromedary camel breeds differing in



Fig 1. Agarose gel electrophoresis for amplification of the KRTAP7 gene in Indian camel breeds: - Lane 1-3: Mewari; Lane 4-6: Kachchhi; Lane M: 100-1500bp NEX-GEN DNA ladder; Lane 7-9: Bikaneri; Lane 10-12: Jaisalmeri



A: Indicate considered sequences of India dromedary camel; N.B. in right represent the accession number and scientific name of representative mammalian species.

Fig 2. Phylogeny tree for Coding Sequences of the *KRTAP7* gene depicting evolutionary relationship between Indian camel and other mammalian species.

hair quality attributes. In our study, the identical consensus nucleotide sequence for the *KRTAP7* gene in four Indian camel breeds indicated the

monomorphic nature of the gene. This monomorphic nature was present in high and low values of fibre diameter and medullation per cent. In addition,



A: Indicate considered sequences of India dromedary camel; N.B. in right represent the accession number and scientific name of representative mammalian species.

Fig 3. Phylogenetic analysis for the KRTAP7 amino acid sequence depicting evolutionary relationship between Indian camel and other mammalian species.



Fig 4. Multiple protein sequence alignment for KRTAP7 protein with different species

considering other associated gene(s) may establish the genetic association of hair fibre traits.

Acknowledgements

The authors thank the Director, ICAR-NRCC, Bikaner for providing all the necessary support for the

Conflict of Interest

No any conflict of interest.

present study. The financial support for the first author

by CVAS, Bikaner, Rajasthan, India with an Institutional fellowship during the work is thankfully acknowledged.

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