Indian Journal of Animal Sciences **95** (10): 940–946, October 2025/Article
https://doi.org/10.56093/ijans.v95i10.155562

SNP characterization of DSG3 gene for high-altitude adaptation in Chaugarkha goat of Kumaon Himalaya

MONICA SINGH[1], APEKSHA[2], INDRASEN CHAUHAN[3]✉, ANUJ CHAUHAN[4], CHHAYA RANI[5], AMIR KUMAR SAMAL[6] and CHANDRAKANTA JANA[7]

ICAR-Indian Veterinary Research Institute, Izatnagar-243 122

Recevied: 26 August 2024; Accepted: 30 January 2026

ABSTRACT

Livestock in high-altitude environments have developed genetic adaptations, including physiological and morphological changes, to thrive in challenging conditions. The DSG3 gene (Desmoglein 3) on chromosome 24 of goats has been identified as a key player in the adaptation of goat breeds at high altitudes. A study focused on DSG3 revealed 27 SNPs variants between lowland and highland goat breeds, with three non-synonymous SNPs (R597E, T595I, and G572S) located in exon 5. To address the gap in knowledge regarding the genetic basis of high-altitude adaptation, this study aimed to characterize non-synonymous SNPs in exon 5 in DSG3 gene by analyzing Sanger sequences from 100 Chaugarkha goats. Sanger sequencing data were analyzed to identify genetic variation and assess the functional impact of non-synonymous SNPs. DNA and amino acid sequences were aligned to the San Clemente goat reference genome (ARS 1.2 Assembly) using MEGA11 with the ClustalW algorithm, and translations were performed using ExPASy. The functional effects of amino acid substitutions were predicted using Mutation Assessor, and protein stability changes were evaluated through structural modeling with the Site-Directed Mutator (SDM) tool. Variants were screened for insertions/deletions and premature stop codons using MEGA11. The potential effects of coding variants on pre-mRNA splicing were assessed using sequence-based splice prediction approaches using Human Splicing Finder, NNSPLICE, and GeneSplicer. Haplotype relationships were inferred using a median-joining network in NETWORK v10. Additionally, 100 diploid Chaugarkha goats were genotyped for three biallelic SNPs, allele frequencies, heterozygosity, and Hardy–Weinberg equilibrium were evaluated using chi-square and exact tests. Analysis revealed two non-synonymous SNPs (G572S and T595I) and one synonymous SNP in exon 5 of Chaugarkha goats. The two SNPs (chr24:g.25794771G>A and chr24:g.25794695C>T) showed complete heterozygosity in all individuals and strong deviation from Hardy–Weinberg equilibrium, whereas a third SNP (chr24:g.25794621G>C) was rare and conformed to equilibrium. Functional prediction analyses indicated that the associated amino acid substitutions (G572S and T595I) do not affect DSG3 protein function, stability, splicing, or structure. Haplotype analysis identified three haplotypes, with one predominant and one rare haplotype defined by a private mutation. Overall, the variants exhibited distinct genetic patterns but no predicted functional impact. In conclusion, the *in silico* tools predicted no drastic impact, despite the occurrence of non-synonymous SNPs in the gene, as this gene plays some of the very important biological roles. The experimental assays are needed to confirm the *in silico* prediction.

Keywords: Chaugarkha goat, DSG3, High altitude adaptation, Sanger sequencing, SNP

Present address: [1]Veterinary Officer, Bihar Veterinary Service, Baisa-854315, Purnia, Bihar, INDIA. [2]Ph.D. Scholar, Animal Genetics Division, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Bareilly, Uttar Pradesh, INDIA. [3]Senior Scientist, Temperate Animal Husbandry Division, ICAR-Indian Veterinary Research Institute, Mukteshwar Campus-263138, Nainital, Uttarakhand, INDIA. [4]Principal Scientist, Livestock Production and Management Section, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Bareilly, Uttar Pradesh, INDIA. [5]Ph.D. Scholar, Animal Genetics Division, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Bareilly, Uttar Pradesh, INDIA. [6]Scientist (SS), Temperate Animal Husbandry Division, ICAR-Indian Veterinary Research Institute, Mukteshwar Campus-263138, Nainital, Uttarakhand, INDIA. [7]Principal Scientist, Animal Pathology Division, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Bareilly, Uttar Pradesh, INDIA. ✉Corresponding author e-mail: indrachauhan55@gmail.com

Goat rearing plays a significant role in livelihood security, serving as an important source of income for poor, marginal farmers and landless people in rural India. The Chaugarkha goat is a medium-sized breed distributed across the Kumaon Himalaya of Uttarakhand and is primarily reared for meat (Jana *et al*. 2020). Its breeding tract spans four mountainous districts of Kumaon Almora, Bageshwar, Champawat, and Pithoragarh as well as some pockets in Nainital district. A specific tract, Chaugarkha Patti, extends from Chitai to Danya villages in Almora, and the breed is eponymously named after this region. The average altitude of Kumaon rises to approximately 7,000 ft above sea level. High-altitude regions are characterized by hypoxic conditions, low temperatures, strong ultraviolet

radiation, and limited feed resources (Billings and Mooney, 1968; Friedrich and Wiener, 2020). The combination of hypoxia, cold temperatures, and scarce feed makes survival in these regions extremely challenging. Livestock inhabiting such harsh environments have evolved multiple adaptive strategies over time, many of which have a genetic basis (Friedrich and Wiener, 2020). Genetic and functional analyses have identified several genes associated with high-altitude adaptation, including EPAS1 and HBB (Gou *et al*. 2014; Fan *et al*. 2015; Song *et al*. 2016), as well as genes enriched in the VEGF (vascular endothelial growth factor) signaling pathway, such as SIRT6, PTEN, AKT1, FGF2, EGFR, MITF, ENPEP, KDR, and CDC42 (Meilin et al. 2020). In addition to these, the DSG3 gene (Desmoglein 3) has been implicated in high-altitude adaptation in goats. DSG genes are primarily expressed in desmosomes, and their encoded proteins are critical for cell-to-cell adhesion, maintaining cellular structural integrity, and stabilizing cells under adverse conditions. In goats, DSG3 is located on chromosome 24, spans approximately 44 kbp, and consists of 16 exons (Kumar *et al*. 2018). The gene is expressed in hair follicles and mucosal epithelium, highlighting its functional significance (Koch *et al*. 1998). The resequencing of the entire DSG3 locus across multiple Tibetan goats revealed three highly differentiated non-synonymous SNPs located in exon 5 that significantly segregate high-altitude Tibetan goats from lowland populations. These exon 5 variants (R597E, T595I, G572S) showed strong allele frequency correlations with altitude adaptation and large measures of population differentiation (F_{ST}), indicating that exon 5 harbours evolutionarily important variation linked to adaptive phenotypes in caprine DSG3 (Kumar *et al*. 2018). The Chaugarkha goat is naturally adapted to the harsh temperate climatic conditions of the Kumaon Himalayan region. To date, only phenotypic characterization of the breed (Jana *et al*. 2020) and genetic analysis using microsatellite markers (Ganie *et al*. 2017) have been reported. No studies have investigated the genetic basis of the adaptation of Chaugarkha goat to the high-altitude, temperate environment of the Kumaon Himalaya. Therefore, the present study was undertaken to genetically characterize the DSG3 gene in Himalayan Chaugarkha goats and to explore its potential role in adaptation to high-altitude conditions.

MATERIALS AND METHODS

Collection of samples: 3-4 ml of blood samples were collected in EDTA coated vacutainers from 100 Chaugarkha goats maintained at Surmane goat farm located at ICAR-Indian Veterinary Research Institute, Mukteshwar, Uttarakhand. The collected blood samples were stored at 4^0C for further processing. For the blood collection, the approval of Institute Animal Ethics Committee was obtained.

DNA extraction: For the DNA extraction from blood sample and preparation of reaction mixture for PCR, Blood-Direct PCR kit from Real Gene™ were used. About 5µl of whole blood sample were placed into a PCR tube and 100µl of DNA prep solution were added. The sample was heated for 10 minutes at 95˚C in a PCR machine. Then, the sample was taken out, mixed a few times, and used for PCR. Any sort of undigested tissue or debris was avoided.

Preparation of PCR reaction mixture and running PCR: PCR reactions were set up and the details of the reagents added to a PCR tube are given in the Table 1. PCR thermal cycling conditions are given in the Table 2.

The gene consisted of 16 exons. The sets of the primer for exon 5 as reported by Kumar *et.al.* 2018 were used to amplify the exon 5. The forward and reverse primer sets were AACATACACGACCTGCTCTGC and AACCCCAACAGCCCTCATAA, respectively. PCR reaction of 20µl volume were made and gradient PCR was run for primer optimization. PCR products were visualized by gel electrophoresis. 1.5% of gel were made, which was stained with ethidium bromide. The 6X loading dye and100 bp marker were used to compare the size of the amplicon.

Gel cutting, gel purification and *sequencing of eluted DNA samples:* The DNA band of desired length were cut from the gel using the UV Transilluminator with the help of scalpel blade, and the gel pieces were transferred to Eppendorf tubes and stored at -20˚C. For gel purification, Gel Extraction Kit (Qiagen, Hilden) was used. The eluted DNA was stored at -20˚C and sequenced using Sanger method of DNA sequencing.

Statistical analyses: For the analysis of Sanger sequences, following three software were used: (1) MEGA11(Tamura *et al*. 2021), (2) ExPASy (Gasteiger *et al*. 2003), and (3) Mutation Assessor (Reva *et al*. 2011). MEGA11 was used for multiple sequence alignment (MSA) of both DNA and amino acid sequence. For multiple sequence alignment, the DNA and amino acid sequences of San Clemente goat (ARS 1.2 Assembly) available in NCBI was used as the reference. ClustalW algorithm was used for multiple

Table 1. Reaction mixture for PCR

Ingredients	Quantity
2X Direct- PCR Mix	20µl
Primers	2µl of both forward and reverse primers
Nuclease Free Water	12µl
Sample	4µl
Total	40µl

Table 2. PCR cycling conditions

Step	Temperature	Time	Cycles
Initial Denature	95^0C	3 min	1
Denature	95^0C	30 Sec	35
Annealing	63^0C	30Sec	35
Extension	72^0C	30Sec	35
Final Extension	72^0C	7min	1
Hold	40^0C	∞	

sequence alignment. For the translation of the DNA sequence, ExPASy was used. The DNA alignments were inspected for the existence of nucleotide substitutions and indels. The translations were performed using the standard genetic code, and amino acid alignments were inspected for the existence of non-synonymous mutations and premature stop codons. To predict whether the amino acid substitution affected protein function or not, Mutation Assessor (available at http://mutationassessor.org.) For functional validation through protein modelling, the functional effects of the variants were assessed by protein modeling using Site-Directed Mutator (SDM) tool (Worth *et al*. 2011), which predicts changes in protein stability ($\Delta\Delta G$) based on structural environment-specific substitution matrices. The effects of coding variants on pre-mRNA splicing were evaluated using Human Splicing Finder (Desmet *et al*. 2009), NNSPLICE (Reese *et al*. 1997), and GeneSplicer (Pertea *et al*. 2001). The effects of coding variants on pre-mRNA splicing were evaluated using sequence-based criteria adapted for non-human species. For each variant, the surrounding exon was analyzed for (i) disruption or creation of canonical splice donor (GT) or acceptor (AG) sites, (ii) potential cryptic splice sites, and (iii) alterations of exonic splicing regulatory elements (ESE/ESS motifs). The haplotype relationships were reconstructed using a median-joining network implemented in NETWORK v10.2.0.0. (Fluxus Technology Ltd.). The analysis was performed using variable sites only, with equal weighting of characters and default settings. Median vectors were included to represent inferred ancestral haplotypes, and the final network was visualized within the NETWORK software. The network was constructed with exon 5 sequences of San Clemente goat extracted from ARS 1.2 Assembly. Finally, a total of 100 diploid animals were genotyped for three biallelic SNPs. Genotype and allele frequencies were calculated for each SNP. Deviations from Hardy–Weinberg equilibrium (HWE) were tested using a chi-square (χ^2) goodness-of-fit test with one degree of freedom when all expected genotype counts were ≥5. For SNPs with low expected genotype counts, an exact Hardy–Weinberg test was applied. Observed heterozygosity (H_o) was calculated as the proportion of heterozygous individuals. Exact tests were conducted using the Hardy Weinberg package in R (Graffelman, 2015).

RESULTS AND DISCUSSION

The primer 5 used in the study was optimized at a temperature 63°C and the amplicon size was 600 bp (Fig.1).

Analysis of Sanger sequences: After multiple sequence alignment of DNA sequences, three SNPs in exon 5 of Chaugarkhagoatrevealedtwo SNPs(chr24:g.25794771G>A and chr24:g.25794695C>T) in all animals and one SNP (chr24:g.25794621G>C) in one animal (Fig.2). However, only two SNPs were non-synonymous (G572S and T595I)

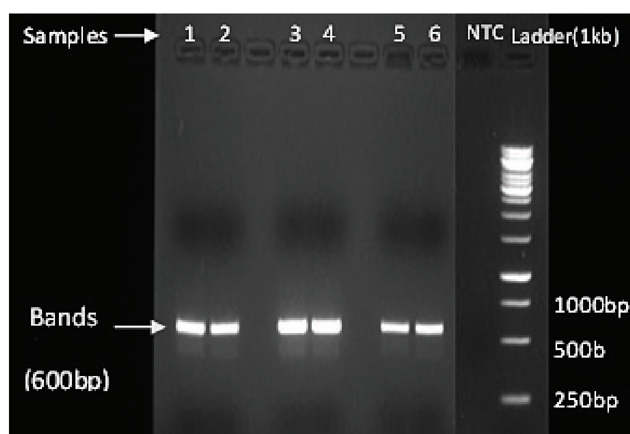

Fig. 1 Agarose gel electrophoresis for amplified product of Exon5 specific to DSG3 gene. Lane 1 to 6: Representative samples indicating positive amplification of 600bp; NTC: Non template control; DNA ladder is of 1kb.

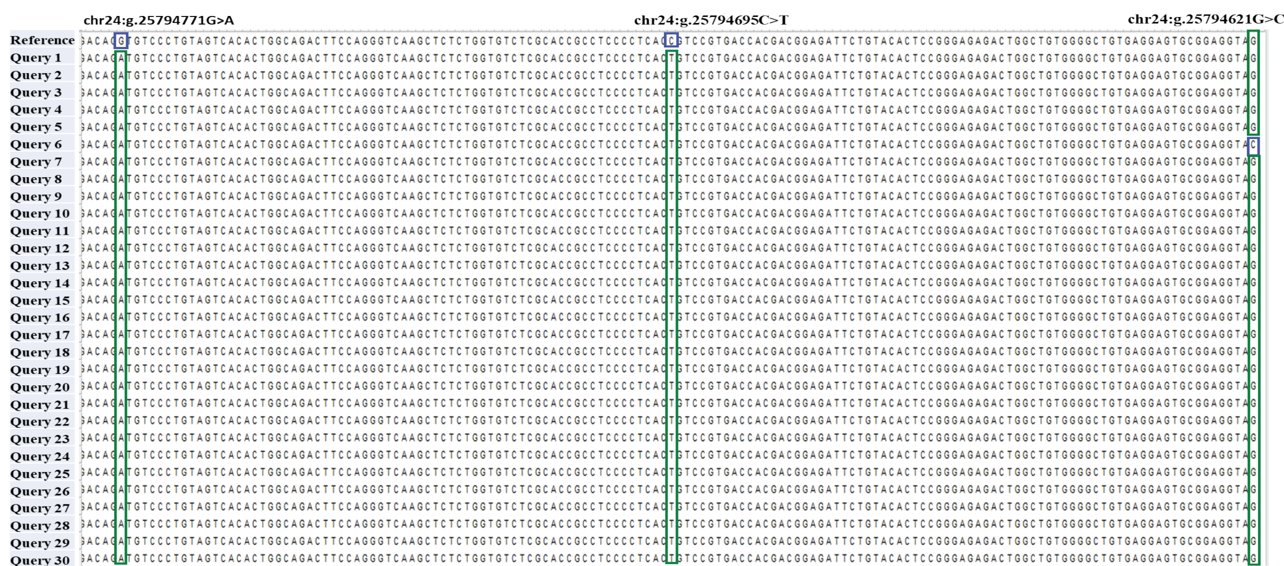

Fig. 2 Multiple sequence alignment of DNA sequences (only first 30 query sequences are shown; Reference sequence is from San Clemente goat)

```
Reference TSALLTAPQPVSPGVYRISVVVTDGEGRRCETPESLTLEVCQCDYRDTCRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 1   TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 2   TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 3   TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 4   TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 5   TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 6   TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 7   TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 8   TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 9   TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 10  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 11  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 12  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 13  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 14  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 15  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 16  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 17  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 18  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 19  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 20  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 21  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 22  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 23  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 24  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 25  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 26  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 27  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 28  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 29  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
Query 30  TSALLTAPQPVSPGVYRISVVVTDSEGRRCETPESLTLEVCQCDYRDICRSAPPTSSPHTIREGSRMGAAAIGLLFLGLLLLL
```

Fig. 3 Multiple Sequence Alignment of Amino Acid Sequences (only first 30 query sequences are shown; Reference sequence is from San Clemente goat)

and one SNP (chr24:g.25794621G>C) was synonymous (Fig.3).

For SNPs chr24:g.25794771G>A and chr24:g.25794695C>T, all individuals were heterozygous, resulting in allele frequencies of 0.5 for each allele. Both SNPs showed strong deviations from Hardy–Weinberg equilibrium ($\chi^2 = 100$, $p < 0.001$), with a complete excess of heterozygotes ($H_o = 1.0$). For SNP chr24:g.25794621G>C, only one heterozygous individual was observed, while the remaining 99 individuals were homozygous for the G allele. The minor allele frequency was 0.005, and observed heterozygosity was 0.01. Due to low expected genotype counts, a χ^2 test was not appropriate. An exact Hardy–Weinberg test showed no deviation from equilibrium (Exact p=1.00) (Table 3).

Predictions of protein functions: From the analysis by Mutation Assessor, it was found that both amino acid substitutions (G572S and T595I) did not impact the function of the DSG3 protein (Table 4).

Further functional validation through protein modelling using Site-Directed Mutator (SDM) tool (Worth *et al.* 2011) predicted no changes in protein stability (ΔΔG) based on structural environment-specific substitution matrices. The variants were not predicted to cause stop-gain mutations and did not introduce insertions or deletions (indels). Neither variant was predicted to disrupt canonical splice donor or acceptor sites, nor to generate novel cryptic splice sites within the analyzed exon sequence. In addition, neither substitution was predicted to cause substantial alteration of exonic splicing enhancer or silencer motifs. Regarding haplotype network, median-joining network analysis identified three haplotypes (H1, H2, and H3).

Table 3. Sample size, allele counts, allele frequencies, Genotypic frequencies, genotype counts (observed and expected), χ^2 test for Hardy–Weinberg equilibrium, and observed heterozygosity for each of the three SNP.

SNP	Sample size (N)	Allele counts (A_1/A_2)	Allele frequencies (p/q)	Genotypic frequencies	Observed genotypes	Expected genotypes under HWE	HWE test	p-value	Observed heterozygosity (Ho)
G>A	100	G=100 / A=100	0.50 / 0.50	GG=0.0/ GA=1.0/ AA=0.0	GG=0/ GA=100/ AA=0	25/50/25	Chi-square	<0.001	1
C>T	100	C=100 / T=100	0.50 / 0.50	CC=0/ CT=1.0/ TT=0	CC=0/ CT=100/ TT=0	25/50/25	Chi-square	<0.001	1
G>C	100	G=199/ C=1	0.995/ 0.005	GG=0.99/ GC=0.01/ CC=0.00	GG=99/ GC=1/ CC=0	99.0025/ 0.995/ 0.0025	Exact	1.0	0.01

Table 4. Predictions of functional impact of amino acid substitutions

	Reference allele	Mutant allele	Amino Acid Change	Predictions on function
SNP1	G	A	G572S	No effect on protein function
SNP2	C	T	T595I	No effect on protein function

Of these, H2 was the predominant haplotype, present in almost all the sequences sharing SNP1 and SNP2, whereas H3 was rare, occurring in a single sequence (Sequence 6) with a unique private mutation, SNP3 (Fig.4).

An amplicon of size 600 bp was obtained for exon 5. DSG3 (Desmoglein 3) as a candidate gene responsible for the high-altitude adaptation was identified for the first time by in Tibetan cashmere goat breeds (Song *et al.* 2016). But, all 16 exons in the goat were not fully covered by this study. This was extended by Kumar *et al*. 2018 through resequencing all the 16 exons of the DSG3 gene in ten Chinese goat populations, where they reported three SNPs in exon 5, namely Chr24:g.25794771T>C, Chr24:g.25794695G>C and Chr24:g.25794694C>T. In the present investigation, three SNPs were found, including two SNPs (Chr24:g. 25794771G>A and Chr24:g.25794695C>T) in all animals and only one SNP (Chr24:g.25794621G>C) in one animal. The SNPs Chr24:g.25794771G>A (in the present study) and Chr24:g.25794771T>C (in Kumar *et al*. 2018) resulted in same non-synonymous mutation (G572S), as they are basically same nucleotide changes (the apparent difference in nucleotide change is due to the use of coding and template strands in the two studies). Similarly, the nucleotide changes Chr24:g.25794695G>C (in Kumar *et al*. 2018) and Chr24:g. 25794695C>T (in the present study) are the same, resulting in T595I non-synonymous mutation. But, SNP Chr24:g.25794621G>C was synonymous. There is a geographical differentiation in the occurrence of the SNPs G572S and T595I with height in goats. In lowland goat populations of China, the non-synonymous mutations G572S and T595I were either rare or absent in the lowest-altitude groups. Their frequencies, however, increased progressively with altitude, reaching high levels in Tibetan goats from high elevations (Kumar *et al*. 2018). As noted above, these SNPs were also observed in the Chaugarkha goat, a breed inhabiting region at an average altitude of approximately 1,500–2,000 meters above sea level. In contrast, the non-synonymous SNP R597E reported by Kumar *et al*. (2018) was not observed. The presence of this variant in some breeds, but not others, likely reflect breed-specific evolutionary pressures, including natural selection and genetic drift, influencing its retention or loss.

It is essential to know the impact of non-synonymous mutations on the function of protein, as non-synonymous mutations result in substitution of one amino acid with other at any particular location in the protein. In the present investigation, from the analysis by Mutation Assessor, it was predicted that the both amino acid substitutions (G572S and T595I) should not impact the function of the DSG3 protein. Moreover, SDM analysis predicted that these variants had negligible effects on protein stability

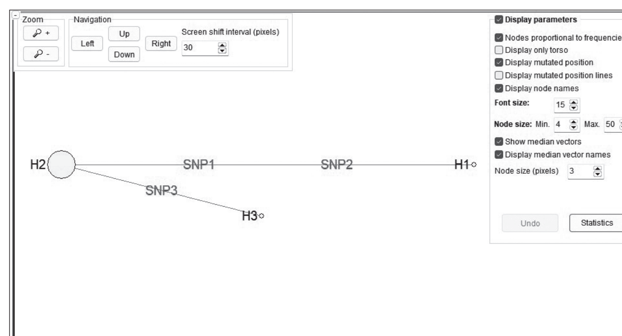

Fig. 4 Median Joining Network. Each node denotes a haplotype, with the circle size proportional to its frequency, and branch lengths representing the number of nucleotide substitutions.

($\Delta\Delta G$ values within the neutral range), suggesting they are unlikely to destabilize the protein structure. Thus, the *in silico* tools predicted no drastic impact, despite the occurrence of non-synonymous SNPs, as this gene is involved in some of the very important biological roles in the vertebrates. The DSG3 gene plays a key role in maintaining the normal structure and function of hair (Song *et al*. 2016; Li *et al*. 2018; Guo *et al*. 2019), skin development (Koch *et al*. 1998; Song *et al*. 2016; Guo *et al*. 2019), additional fiber growth in cashmere goats (Kumar *et al*. 2018), and cell-to-cell adhesion of keratinocytes in the basal and suprabasal layers of stratified squamous epithelia (Kumar *et al*. 2018). It has also been associated with lighter skin pigmentation in Europeans, which contributes to high-altitude adaptation (Huerta-Sanchez *et al*. 2013). These functions are vital for survival at high altitudes, as hair growth and normal skin function help animals withstand low temperatures and protect against strong UV radiation. DSG3 is also important for normal lung and vascular system function. during the early phase of acute hypobaric hypoxia in rats, DSG3 is highly upregulated (Sharma *et al*. 2015). Furthermore, Pulkkinen *et al*. (2002) reported that homozygous deletion of DSG3 in mice causes a blistering phenotype due to loss of keratinocyte adhesion. The experimental assays are needed to confirm the impact of the non-synonymous SNPs on protein function. In silico splice analysis indicated that no variant was likely to affect canonical or cryptic splice sites, supporting a low probability of splicing disruption for the variants. Further, the variants were not predicted to produce stop-gain effects and none of the variants resulted in indels. Median-joining network analysis identified three haplotypes (H1–H3), with H2 predominating in 28 sequences sharing SNP1 and SNP2. H3 was rare, occurring in only a single sequence with a unique private mutation (SNP3). These results indicate that while common haplotypes dominate the population,

rare variants contribute to overall genetic diversity. SNPs Chr24:g. 25794771G>A and Chr24:g.25794695C>T were heterozygous in all individuals, showing complete heterozygote excess (Ho = 1.0) and strong deviation from Hardy–Weinberg equilibrium (χ^2 = 100, p < 0.001). SNP Chr24:g.25794621G>C was nearly fixed for the G allele, with only one heterozygote, and showed no deviation from equilibrium (exact HWE p=1.00).

This study investigated the SNPs in the exon 5 of the DSG3 gene in Chaugarkha goat. This the first time this kind of investigation has been done in Chaugarkha goat. Two SNPs (Chr24:g. 25794771G>A and Chr24:g.25794695C>T) were found in all animals and only one SNP (Chr24:g.25794621G>C) was found in one animal. However, of the three SNPs, only two SNPs were non-synonymous (G572S and T595I) and one SNP Chr24:g.25794621G>C was found to be synonymous. From the functional analysis of the impact of the non-synonymous mutations, it was found that both amino acid substitutions (G572S and T595I) did not impact the function of the DSG3 protein. Other analyses predicted that the identified variants do not affect protein stability, splicing, or create indels and stop-gain mutations. Haplotype analysis revealed three haplotypes, with H2 predominant and H3 rare, carrying a private mutation. As DSG3 gene plays some of the very important biological roles, we can conclude that the *in silico* tools predicted no drastic impact, despite the occurrence of non-synonymous SNPs. Further experimental assays are needed for the confirmation of the impact of the non-synonymous SNPs on protein function.

ACKNOWLEDGEMENTS

Authors would like to acknowledge the guidance and inspiration by Joint Director, ICAR-Indian Veterinary Research Institute, Mukteshwar. For the laboratory works, equipments and facilities from ICAR-National Institute on Foot & Mouth Disease, Mukteshwar (formerly ICAR-Project Directorate on Foot & Mouth Disease, Mukteshwar) were used. This is duly acknowledged. The facilities provided under All India Coordinated Research Project on Goat Improvement (Himalayan Goat unit) is also acknowledged.

REFERENCES

Billings W D and Mooney H A. 1968. The ecology of arctic and alpine plants. *Biological Reviews* **43**(4): 481–529.

Desmet FO, Hamroun D, Lalande M, Collod-Béroud G, Claustres M and Béroud C. 2009. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. *Nucleic Acids Research*.**37**(9): e67. doi:10.1093/nar/gkp215

Fan R, Liu F, Wu H, Wu S, Zhu C, Li Y, Wang G and Zhang Y. 2015. A positive correlation between elevated altitude and frequency of mutant alleles at the EPAS1 and HBB loci in Chinese indigenous dogs. *Journal of Genetics and Genomics* **42**:173–177. doi: 10.1016/j.jgg.2015.02.006

Fluxus Engineering 2020. Network 10 (Version 10.2.0.0). Fluxus Engineering. Retrieved from http://www.fluxus engineering. com/sharenet.htm fluxus-engineering.com

Friedrich J and Wiener P. 2020. Selection signatures for high-altitude adaptation in ruminants. *Animal Genetics* **51**(2): 157–65.

Ganie Z A, Kumar D, Sundip K, Shahi B N and Ghosh A K. 2017. Genetic diversity analysis of Chaugarkha goat using microsatellite markers. *Indian Journal of Animal Sciences* **87**(1): 122–24.

Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel R D and Bairoch A. 2003. ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Research* **31**: 3784–88.

Graffelman J. 2015. Exploring Diallelic Genetic Markers: The HardyWeinberg Package. Journal of Statistical Software, **64**(3): 1–23. https://doi.org/10.18637/jss.v064.i03

Gou X, Wang Z, Li N, Qiu F, Xu Z E, Yan D, Yang S, Jia J, Kong X, Wei Z and Lu S. 2014. Whole-genome sequencing of six dog breeds from continuous altitudes reveals adaptation to high-altitude hypoxia. *Genome Research* **24**(8): 1308–15.

Guo J, Zhong J, Li L, Zhong T, Wang L, Song T and Zhang H. 2019. Comparative genome analyses reveal the unique genetic composition and selection signals underlying the phenotypic characteristics of three Chinese domestic goat breeds. *Genetics Selection Evolution* **51**(1): 70. https://doi.org/10.1186/s12711-019-0512-4.

Huerta-Sanchez E, Degiorgio M, Pagani L, Tarekegn A, Ekong R and Antao T. 2013. Genetic signatures reveal high-altitude adaptation in a set of Ethiopian populations. *Molecular Biology and Evolution* **30**: 1877–88. doi: 10.1093/molbev/mst089

Jana C, Gurav A, Gautam S, Singh H, Dangi S S and Sankar M. 2020. Phenotypic and physiological characteristics of Chaugarkha goats of Uttarakhand. *Indian Journal of Small Ruminants* **26**(1): 120–24.

Koch P J, Mahoney M G, Cotsarelis G, Rothenberger K, Lavker R M, Stanley J R. 1998. Desmoglein 3 anchors telogen hair in the follicle. *Journal of Cell Science* **111**(17): 2529–37.

Kumar C, Song S, Jiang L, He X, Zhao Q, Pu Y, Malhi K K, Kamboh A A and Ma Y. 2018. Sequence characterization of DSG3 gene to know its role in high-altitude hypoxia adaptation in the Chinese Cashmere goat. *Frontiers in Genetics* **9**: 553. doi: 10.3389/fgene.2018.00553

Li Y, Zhou G, Zhang R, Guo J, Li C, Martin G, Chen Y and Wang X. 2018. Comparative proteomic analyses using iTRAQ-labeling provides insights into fibre diversity in sheep and goats. *Journal of Proteomics* **172**: 82–8.

Meilin J, Lu J, Fei X, Lu Z, Quan K, Liu Y, Chu M, Di R, Wei C and Wang H. 2020. Selection Signatures Analysis Reveals Genes Associated with High-Altitude Adaptation in Tibetan Goats from Nagqu, Tibet. *Animals* **10**(9):1599. doi: 10.3390/ani10091599.

Pertea M, Lin X and Salzberg SL. 200. GeneSplicer: a new computational method for splice site prediction. Nucleic Acids Research **29**(5): 1185–1190. doi:10.1093/nar/29.5.1185

Pulkkinen L. Choi Y W, Simpson A, Montagutelli X, Sunderg J, Uitto J and Mahoney M G. 2002. Loss of cell adhesion in Dsg3bal–Pas mice with homozygous deletion mutation (2079del14) in the desmoglein 3 gene. *Journal of Investigative Dermatology* **119**:1237–43.

Reese MG, Eeckman FH, Kulp D, Haussler D. 1997. Improved splice site detection in Genie. *Journal of Computational Biology*. **4**(3):311–24.

Reva B, Antipin Y and Sander C. 2011. Predicting the Functional Impact of Protein Mutations: Application to Cancer Genomics. *Nucleic Acids Research* **39**(17): e118, https://doi.org/10.1093/

nar/gkr407. Availability: http://mutationassessor.org.

Song S, Yao N, Yang M, Liu X, Dong K, Zhao Q, Pu Y, He X, Guan W, Yang N and Ma Y. 2016. Exome sequencing reveals genetic differentiation due to high-altitude adaptation in the Tibetan cashmere goat (*Capra hircus*). *BMC genomics* **17**(1): 1–2.

Sharma P, Bansal A and Sharma P C. 2015. RNA-seq-based transcriptome profiling reveals differential gene expression in the lungs of sprague-dawley rats during early-phase acute hypobaric hypoxia. *Molecular Genetics and Genomics* **290**: 2225–2240. doi: 10.1007/s00438-015-1064-0.

Tamura K, Stecher G and Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution* https://doi.org/10.1093/molbev/msab120.

Worth CL, Preissner R, Blundell TL. 2011. SDM—a server for predicting effects of mutations on protein stability and malfunction. *Nucleic Acids Research* **39**: W215–W222. doi:10.1093/nar/gkr363.