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Association Analysis of LCORL Genetic Variant (rs657074013) with Wither-Height in Pakistani Goats

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ABSTRACT

The goat stands as one of the most valued and economically efficient domestic animals, contributing significantly to human welfare through the provision of meat, milk, fiber, skin, and manure. Notably, the initial two production traits are contingent upon the physical attributes of the animals, exemplified by characteristics such as large body size and skeletal frame. Earlier (GWA) studies, employing high-density arrays, have revealed a noteworthy association of various variants located at the boundaries of NACPG and LCORL genes across diverse livestock species. **Objective:** To investigate the genetic variability/association of rs657074013A>AT variant of LCORL gene within diverse goats from Pakistan. **Methods:** ARMS-PCR genotyping technique was used where, a total of 51 goats belongs to diverse breeds were screened with allele-specific set of primers. **Results:** Current study showed that 27% sampled population is homozygous wild-type (A/A), 24% is homozygous-mutant (AT/AT) and 49% is heterozygous (A/AT) with a significant genetic association $x2$ *p-value* = 9.60×10^{-5} using PLINK. Hardy-Weinberg Equilibrium revealed that overall sampled population obeys the principle with $x2$ (2, N = 51) = 0.046, p = 0.9730. Furthermore, alternative allele frequencies (AAF) of 0.68 and 0.28 were also observed within cases and control cohorts respectively along with an odds-ratio of 5.242 which depicts the AAF is ~5 times higher in cases vs controls. **Conclusions:** In summary, this pilot-scale study has advanced our genomic understanding by examining the variability and association of this LCORL variant (c.828_829insA) within the Pakistani goat population. The insights gained hold promise for enhancing this economically crucial trait through the implementation of marker-assisted breeding strategies in this particular species and warrant further exploration in other livestock species to broaden our understanding and potential applications.

INTRODUCTION

The Capra hircus, commonly known as domestic goat, is a member of the animal family Bovidae. Approximately, 40 animal species are domesticated all over the world [1], and goats are one of the oldest species being domesticated as a vibrant producer of meat, milk, hair, fiber and skin. Goats are not only bred for these aforementioned important traits, but also for a variety of additional purposes such as aesthetic and environmental friendly livestock. Studies shows that domestication of goats commenced over 11,000 years ago from wild bezoar (Capra aegagrus) populations [2-4]. There are 27 goat breeds in Pakistan. Annually, goats produces ~275 thousand tonnes of meat, ~851 thousand tonnes of milk, ~25 million skins and ~21.4 thousand tonnes of hair and thus play an important role in the economy nationwide [5]. Goat meat is one of the most desired around the world. Due to rising demand of goat meat, farmers are interested in raising and breeding goats for more protein yield. The expansion of goat-products industry is dependent on the growth and well-being of goats itself. Body measurement traits such as body size/stature and development significantly affects the performance and the yield procured from the goats. So, body-height and skeletal-frame is one of the targeted trait to improve meat and milk production through adopting marker-assisted breeding strategies.The main factor that control animals' height and skeletal stature is their genetic

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makeup. There are plethora of genes that control body-height and skeletal-frame in different animal species, such as the Sperm Associated Antigen-17 (SPAG17) and Pleomorphic Adenocarcinoma (PLAG1) [6, 7]. But the gene that has been the most studied and observed to be strongly associated with frame-size of goats is the Ligand Dependent Receptor Co-repressor Like (LCORL), also known as the Mblk-1-related protein [8]. LCORL encodes a transcriptional factor that has been linked to body-stature and skeletal-frame. In large-sized goats, the truncation may interfere with LCORL's transcription factor binding to its target, because the N-or C-terminal region of this protein is removed by proteolysis, premature protein elongation occurs due to the gain of stop codon and a nonsense mutation in the structured gene, it also has a function in the process of spermatogenesis. In Capra hircus, the LCORL gene has been mapped on Chr.6 (NC_030813.1) genome assembly ARS1.2 (GCF_001704415.2) having 8 exons in total [9]. It has been identified in previous studies that the c.828_829insA locus at genomic position 6:37928641, (rs657074013A>AT) with r.1225 nucleotide position of transcript ID: (XM_018049322.1) located on exon 7 (Figure 4), which is found highly variable with goat-height and skeletal-frame [8]. The aim of the current study is to evaluate the association of the aforementioned variant with heighted phenotypic trait in Pakistani goat population for improving the overall meat and milk production in this valued species, at the same time, the subject trait may be improved by investigating the same variant in other livestocks species.

METHODS

Sample collection and DNA extraction

Blood samples of 51 goats were collected to evaluate the genetic association between the LCORL variant (rs657074013A>AT) with their wither-height and skeletal-frame. For sampling, two goat groups were made, one is categorized as a heighted-cohort (n = 25, wither-height ≥ 36") and the other one as a control-cohort (n = 26, wither-height < 36") of the age bracket 1.5-2 years (Figure1). EDTA vacutainers were used to collect and store blood samples at -20°C till further usage. GDSBio (https://www.gdsbio.com/en/) genomic DNA extraction kit was used to extract DNA from goat blood samples by following the manufacturer's instructions.

Figure 1: A few sampled goats, upper row animals are of wither-height ≥ 36", while lower ones are below this threshold.

Primer designing

ARMS-PCR primers were designed using OligoCalc software(http://biotools.nubic.northwestern.edu/OligoCalc.html) for the amplification of wild-type and mutant allele at (c.828_829insA) locus in goats against the transcript ID: XM_018049322.1. A total of five primers were designed, which were labelled as reverse common, forward normal and forward mutant ARMS primers with amplicon-size of 300bp. Similarly, two internal control (IC) primers were also designed to amplify a region for PCR fidelity (Table 1).

Table 1: Details of primers' sequences.

ARMS/ Internal Control (IC)	Sequence (5'-3')	Tm (°C)	Length (bp)	Product Size (bp)
Reverse Common	AATCTTTAATACAGACTGGCAGAG	60.3	24	300
Forward Normal	AGCTACTAAAATGGAAAAAGGAAGAT	60.1	26	
Forward Mutant	AGCTACTAAAATGGAAAAAGGAAGAA	60.1	26	
Forward (IC)	GCATTGCTAGTCTGCTCCATTA	60.1	22	714
Reverse (IC)	GTCGACTGTGAAGAATCAAGAG	60.1	22	

DNA amplification

The ARMS-PCR reaction was carried out using a SimpliAmp thermal cycler (Applied Biosystems). Two PCR reactions were carried-out separately with each sample having normal (N) and mutant (M) type ARMS allele specific forward primer along with reverse common primer. Simultaneously, two regular primers were also used to amplify the genomic region as an internal control in few of the random samples. A total of 16µL of the reaction mixture was prepared consisting 1µL of 50ng/µL genomic DNA, 10mM of each primer, 0.05IU/µL of Taq polymerase, 2.5mM MgCl2, 2.5mM dNTPs, 1x Taq buffer and PCR-grade water. The PCR protocol was adopted with 5-minutes of initial denaturation at 95°C followed by 30 cycles of denaturation (95°C for 45sec.), annealing (57°C for 30sec.), extension (72°C for 45sec.) with the final extension at 72°C for 10-minutes and stored at 4°C (Figure2).

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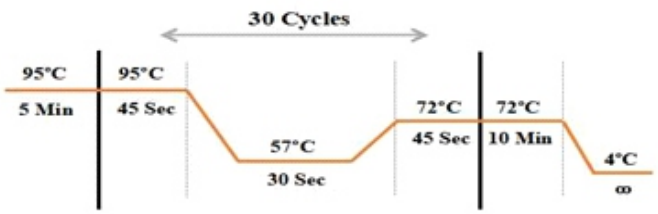

Figure 2: Thermal cyclic conditions of ARMS-PCR

Statistical analysis

HWE was used to calculate the observed & expected allelic and genotypic frequencies by obeying $p2 + 2pq + q2=1$ equation and further Chi-square analysis was also conducted using $x2 = \sum \frac{(0-E)2}{E}$ equation to calculate the p-value to check whether our sampled population is in accoradance with HWE or not. Secondly, PLINK data analysis toolset was used to evaluate the association of subject variant with the wither-height phenotype after screening the subject locus (rs657074013A>AT) in the sampled population of 51 goats. AAF in cases and controls along with p-value and odds-ratio were also calculated in overall goat population.

RESULTS

In the current study, LCORL variant (rs657074013A>AT) showed the variability in Pakistani goat population, which is in accordance to the other goat populations of the world, and depicts that, this Chr.6 locus 6:37928641 is under-selection in large-sized Pakistani goat breeds. Genotyping results are shown in (Figure 3).

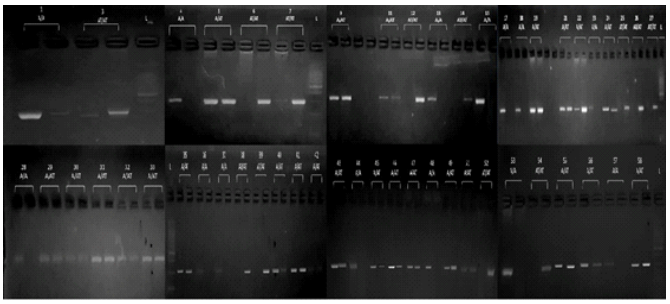

Figure 3: ARMS-PCR amplification of targeted variant within 51 sampled goats

A total of 51 samples were genotyped (cases=25, wither-height ≥36") and (controls=26, wither-height<36"). After experimental and statistical analysis, it was concluded that, there are 2 homozygous wild-type (A/A), 11 homozygous-mutant (AT/AT) and 12 heterozygous (A/AT) individuals observed among the heighted cohort. Similarly, 12, 01 and 13 goats are homozygous wild-type, homozygous-mutant and heterozygous respectively in the control group. Hence, the overall genotypic frequency of homozygous wild-type in our sampled population is 0.27 (27%), homozygous-mutant is 0.24 (24%) and heterozygousis 0.49 (49%). Subsequently, both A/AT alleles frequencies were calculated as 0.52 and 0.48 respectively in oveall population. Thereafter,(*HWE*) Chi-square analysis was conducted to verify, whether our sampled population is obeying this principle or not with the following outcomes of$x2$ (, $N = 51$) = 0.0546,$p = 0.9730$ which manifests that our population is in accordance with the *HWE* equilibrium as the *p*-value is above the set threshold CI of 0.05, so accepting our null-hypothesis of observing *HWE*. Moreover, genetic association analysis was conducted using PLINK data analysis toolset which demonstrated that the AAF are 0.68 & 0.28 within our cases & control cohorts having $x2$ statistics value of 15.65 and *p*-value = $9.60x10^{-5}$ 5showing a significant association of the screened variant with wither-height phenotype in Pakistani goats. Likewise, 5.242 odds-ratio (OR) was also observed showing the prevelance of mutant allele is almost ~5-times heigher in cases vs controls (Table 2).

Table 2: Association of subject variant with goat wither-height from Pakistan

Sampl	Chr. Position	cDNA variant XM_ 018049322.1	Protein variant XP_ 017904811.1	Genotypic frequency			AAF		p-value (OR)
				Homo-wild (#/%)	Hetero- (#/%)	Homo-mutant (#/%)	Case	Cont	
51	6:37928641	r.1225T/TA c.828_829insA	p.Ser277Ilefs*38	14/27	25/49	12/24	0.68	0.28	9.60×10-5 (5.242)

This single base insertion variant of c.828-829insA causes frame-shift in LCORL protein and alters Serine amino acid (aa) to Isoleucine at 277th position in this polypeptide which terminated this protein at 38th position after 277th (aa) and resulted in 314 (aa) truncated LCORL potein instead of its wild-type protein of 1864 (aa). This frame-shift mutation truncated almost ~83% of the wild-type open reading frame due to gain of a premature stop codon at (ser277Ilefs*38) [8, 10]. This truncation may be responsible for the goat's height and appeared as gain/loss of function mutation. Further, fuctional genetics studies are still needed to confirm and validate this postulated hypothsis.

DISCUSSION

The central importance and rising demand of meat is undeniable in the present world. Meat consumption has taken on a new vehemence especially in islamic countries such as Pakistan, where the goat meat is highly preffered and an essential part of the diet of the masses. Moreover, goat milk is consumed due to religious reference among the muslim communities with health benefits. Due to these reasons, goat rearing in Pakistan is a highly profitable

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business and farmers are greatly interested in the better performance of their herds [5]. So, the wither-height and large stature trait chosen in this study to meet the rising demand of goat meat in Pakistan. According to one of the genome-wide genomic selection signature study, the LCORL gene variant rs657074013A>AT on chr.6 was identified as the most relevant quantitative trait locus for height in goats [8]. The molecular mechanism underlying, how the LCORL locus modulates body-size and height across several mammalian species is yet not clear [8]. In the current investigation, we tried to explore the variability of this locus in our indigenous goats that how the genetic variation influenced the subject trait. Our findings revealed that there are only 2 & 12-homozygous wild-type, 11 & 01 homozygous-mutant and 12 & 13 goats are heterozygous in the cases and controls respectively, which depicts the moderate fixation of mutant allele/genotype in the cases while heterozygous individuals are almost same in both groups that further shows the random mating and locus hitchhiking in the population. Moreover, multiple sequence alignment of the subject locus is alos performed in nine mammalian species to check the conservency status of the locus which are showing (T) nucleotide in goat as compare to (G) in horse, pig, humans and cattle while (C) in mouse (Figure 4).

Figure 4: Multiple sequence alignment of genotyped variant in different species A), Genomic location of the subject variant and LCORL protein structure are shown B)

Previous studies have enlightened that PLAG1/LCORL is not only associated with body-stature in goats but in several other species as well e.g., cattle, sheep, horses, dogs, pigs, donkey and humans [7, 11-17]. In 2017, a study from China revealed the association of LCORL gene with the bovine development and carcass traits [6]. Two other studies in 2019-20 found LCORL contributes in body-height and stature in sheep as well [12, 18]. Two other studies showed that the LCORL/NCAPG locus have significant contribution in withers height of horses [19, 20]. Furthermore, the whole genome sequencing of Canids revealed many genomic regions sorrounding LCORL affect the dog's skeletal-frame and its other morphological traits [14]. A study on domestic pigs in 2012 also exhibited selection signatures harbor LCORL gene [15]. Similarly, a recent analysis in China found that the donkey's body-size has also been attributed to the 3:112664848 locus in LCORL [11]. So, the current study provided the enough evidence that the same gene locus found variable and associated with the large-sized Pakistani goats which is in conformity to the other animal species of the world.

CONCLUSIONS

The *LCORL* gene variant (rs657074013A/AT) demonstrates a significant association with wither-height in Pakistani goats, as evidenced by a p-value of 9.60×10-5 using the PLINK χ2 association test. To substantiate these findings, further validation through genetic functional studies is warranted. Additionally, considering the potential implications, marker-assisted breeding strategies could be considered to enhance the height and stature of goat herds in Pakistan.

Authors Contribution

Conceptualization: RS
Methodology: AK, HA, HA, MA, RS
Formal analysis: RS
Writing-review and editing: RS, AK, HA, HA, MA

All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

Preprint available :https://doi.org/10.1101/2022.10.09.511453

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REFERENCES

[1] Ahmad HI, Ahmad MJ, Jabbir F, Ahmar S, Ahmad N, Elokil AA, et al. The domestication makeup: Evolution, survival, and challenges. Frontiers in Ecology and Evolution. 2020 May; 8: 103. doi: 10.3389/fevo.2020.00103.

[2] Zheng Z, Wang X, Li M, Li Y, Yang Z, Wang X, et al. The origin of domestication genes in goats. Science Advances. 2020 May; 6(21): eaaz5216. doi: 10.1126/sciadv.aaz5216.

[3] Colli L, Lancioni H, Cardinali I, Olivieri A, Capodiferro MR, Pellecchia M, et al. Whole mitochondrial genomes unveil the impact of domestication on goat

DOI: https://doi.org/10.54393/fbt.v3i02.46

matrilineal variability. BMC Genomics. 2015 Dec; 16(1): 1-2. doi: 10.1186/s12864-015-2342-2.

[4] Pereira F and Amorim A. Origin and spread of goat pastoralism. EncyclopediaofLifeSciences(ELS). 2010 Sep: 1-10. doi: 10.1002/9780470015902.a0022864.

[5] Iqbal A, Khan BB, Tariq M, Mirza MA. Goat-A potential dairy animal: Present and future prospects. Pakistan Journal of Agricultural Sciences. 2008 Jan; 45(2): 227-30.

[6] Zhang S, Jiang E, Wang K, Zhang Y, Yan H, Qu L, et al. Two insertion/deletion variants within SPAG17 gene are associated with goat body measurement traits. Animals. 2019 Jun; 9(6): 379. doi: 10.3390/ani9060379.

[7] Zhong JL, Xu JW, Wang J, Wen YF, Niu H, Zheng L, et al. A novel SNP of PLAG1 gene and its association with growth traits in Chinese cattle. Gene. 2019 Mar; 689: 166-71. doi: 10.1016/j.gene.2018.12.018.

[8] Saif R, Henkel J, Jagannathan V, Drögemüller C, Flury C, Leeb T. The LCORL locus is under selection in large-sized Pakistani goat breeds. Genes. 2020 Feb; 11(2): 168. doi: 10.3390/genes11020168.

[9] Han YJ, Chen Y, Liu Y, Liu XL. Sequence variants of the LCORL gene and its association with growth and carcass traits in Qinchuan cattle in China. Journal of Genetics. 2017 Mar; 96: 9-17. doi: 10.1007/s12041-016-0732-0.

[10] Graber JK, Signer-Hasler H, Burren A, Drögemüller C. Evaluation of truncating variants in the LCORL gene in relation to body size of goats from Switzerland. Animal Genetics. 2022 Apr; 53(2): 237. doi: 10.1111/age.13177.

[11] Liu Y, Li H, Wang M, Zhang X, Yang L, Zhao C, et al. Genetic architectures and selection signatures of body height in Chinese indigenous donkeys revealed by next-generation sequencing. Animal Genetics. 2022 Aug; 53(4): 487-97. doi: 10.1111/age.13211.

[12] Posbergh CJ and Huson HJ. All sheeps and sizes: a genetic investigation of mature body size across sheep breeds reveals a polygenic nature. Animal Genetics. 2021 Feb; 52(1): 99-107. doi: 10.1111/age.13016.

[13] Staiger EA, Al Abri MA, Pflug KM, Kalla SE, Ainsworth DM, Miller D, et al. Skeletal variation in Tennessee Walking Horses maps to the LCORL/NCAPG gene region. Physiological Genomics. 2016 May; 48(5): 325-35. doi: 10.1152/physiolgenomics.00100.2015.

[14] Plassais J, Kim J, Davis BW, Karyadi DM, Hogan AN, Harris AC, et al. Whole genome sequencing of canids reveals genomic regions under selection and variants influencing morphology. Nature Communications. 2019 Apr; 10(1): 1489. doi: 10.1038/s41467-019-09373-w.

[15] Rubin CJ, Megens HJ, Barrio AM, Maqbool K, Sayyab S, Schwochow D, et al. Strong signatures of selection in the domestic pig genome. Proceedings of the National Academy of Sciences. 2012 Nov; 109(48): 19529-36. doi: 10.1073/pnas.1217149109.

[16] Takasuga A. PLAG1 and NCAPG-LCORL in livestock. Animal Science Journal. 2016 Feb; 87(2): 159-67. doi: 10.1111/asj.12417.

[17] McEvoy BP and Visscher PM. Genetics of human height. Economics & Human Biology. 2009 Dec; 7(3): 294-306. doi: 10.1016/j.ehb.2009.09.005.

[18] La Y, Zhang X, Li F, Zhang D, Li C, Mo F, et al. Molecular characterization and expression of SPP1, LAP3 and LCORL and their association with growth traits in sheep. Genes. 2019 Aug; 10(8): 616. doi: 10.3390/genes10080616.

[19] Tetens J, Widmann P, Kühn C, Thaller G. A genome-wide association study indicates LCORL/NCAPG as a candidate locus for withers height in G erman W armblood horses. Animal Genetics. 2013 Aug; 44(4): 467-71. doi: 10.1111/age.12031.

[20] Mostafavi A, Fozi MA, Koshkooieh AE, Mohammadabadi M, Babenko OI, Klopenko NI. Effect of LCORL gene polymorphism on body size traits in horse populations. Acta Scientiarum. Animal Sciences. 2019 Oct; 42: e47483. doi: 10.4025/actascianimsci.v42i1.47483.