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SINGLE NUCLEOTIDE POLYMORPHISMS ASSOCIATED WITH THE MEAT PRODUCTION IN BALOCHISTANI GOAT BREEDS

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Abstract

Small ruminant farming is a common practice and one of the major sources of livelihood of the rural population of Balochistan. Goat farming has been a common practice throughout the ages for milk, meat, hair, mohair and leather production. Pakistan is the 4th largest goat producing country and Balochistan contributing 22% of the total goat population of the country. Due to its low-fat content, goat meat is healthier compared to beef and mutton. CAST a protein coding gene, has been reported to influence the postmortem tenderization of meat through protein degradation in muscles and considered one of the candidate genes to study meat quality trait in ruminants. The aim of the current study was to investigate CAST gene variants in selected goat breeds under study and its association to meat quality and production and suggest developing plans for future selection of the breeds based on their genetic characterization for the associated traits. The goat breeds of two districts of Balochistan were selected for genetic analysis. The sequencing results revealed 5 single nucleotide polymorphisms including 2 frameshift variations c.290-91delA identified in 70% local and 20% cross breed whereas c.322-25insA at exon 5 identified in 60 % of the local breed, one copy number variant including c.322-25insA and heterozygous missense variation c.326G>T(p.R109I) at exon 5 in 60 % samples of lasi breed and 20% of mixed breed, and 2 splice site variations c.630+13G>A in all breeds under study and c.699-701insG at exon/intron 10 boundary identified in 53% in the local breed.

Keywords: *Calpastatin, Crossbreed, Meat Quality, Small Ruminants, SNPs*

INTRODUCTION

Natural selection is the natural phenomenon through which individuals adapt new and prevailing environmental conditions to make their survival possible. But in the field of animal breeding and genetics various selection and breeding strategies have been and are being adapted by the researchers/farmers to improve the desirable qualities and products in the farm animals (1-3). Domestication of farm animals was made about 12000 years ago and the first domesticated animals were sheep and goat domesticated about 9000-12000 years ago. Goats are among the earliest domesticated ruminants, originating from the wild species; *C. hircus, C. caucasica*, *C. ibex*, *C. falconeri* and *C. pyrenacia* (4-9).

Balochistan is the largest province of Pakistan covering ~44 % of the total area of the country with least human population. More than 70% of the Balochistani population is of rural type and living below the poverty line. Small ruminant farming is a common practice among rural population and one of the major sources of livelihood among the rural population. Balochistan contributing ~48% of the total sheep, ~22% goat, and >44% of the camel population of the country. All these animals are kept and raised in rangeland that is comprised of >90% of the total geography of the province.

Goat farming has been a common practice throughout the ages for milk, meat, hair, mohair wool and leather production. In modern era Asia and Africa have the highest population ~59 and ~37% of goats raised worldwide, and Pakistan is the 4th largest goat producing country with ~66million heads followed by China, India and Nigeria with 188, 133 and 71 million heads (10-11). Goat meat is healthier compared to beef and mutton due to low fat content (12). *CAST* gene is a protein coding gene located on chromosome 7 (14,437,312-14,567,828) in goat. *CAST* gene influences the postmortem tenderization of meat through protein degradation in muscles by synthesizing a calcium-dependent cysteine protease (Calpain) and considered one of the candidate genes to study meat quality trait in ruminants and poultry (13-20). Current study was performed to identify and characterize genetically and phenotypically the goat breeds with meat production traits raised in district Khuzdar and Lasbela of Balochistan by analyzing the *CAST* gene variation analysis within and among the goat breeds under study.

MATERIALS AND METHODS

The farmers of the selected districts were visited. Animals were observed phenotypically and classified as local, lasi and mixed/cross breeds based on their morphological characteristics. Venous blood (5ml) from jugular vein was collected in heparinized tubes from 45 randomly selected animals (as a pilot study and limited funds available for genetic analysis) 15 from each selected breed. DNA was extracted from the blood samples through a non-organic DNA extraction protocol (Already in practice in the molecular genetics lab, FV&AS, LUAWMS) using tris EDTA (T.E) cell lysis buffer for washing the blood samples, protein digestion was carried out by using Proteinase K along with tris natrium EDTA (TNE) buffer and sodium dodecyl sulphate (SDS) and separated by using supersaturated sodium chloride (NaCl). DNA was precipitated by using chilled isopropanol, washed by 70% ethanol, dried at room temperature, and dissolved in PCR graded RNase free water. DNA quantification was done by spectrophotometer and all the DNA samples were brought into equal concentration 25 µg/µl. Due to limited funding primers were designed through amplifx (2.1.1) software and got synthesized only against the selected coding exons (1-10) of the *CAST* gene. DNA samples were prepared in a 25 µl PCR reaction mixture including Taq Master Mix buffer (12.5 µl) Primer forward and reverse (3 µl) each, DNA sample (2 µl), 4.5 µl H^2O (PCR graded, RNase free) and amplified using thermal cycler (T100, BIORAD). After gel electrophoresis, PCR products observed under UV light and processed for ExoSAP-IT clean up and incubated at 37°C for 15 minutes and 80°C for 15 minutes in thermal cycler. Sequencing PCR performed using 10 µl sequencing reaction mixture containing 5X sequencing buffer (2.5 µl), 1 µl forward primer, 0.5 µl Big Dye (v 3.1), 1 µl PCR product and 5 µl PCR graded RNase free H^2O. Sequencing PCR products were precipitated with precipitation mix, washed with 70% ethanol, soaked, and were dissolved in 10 µl each H^2O and HiDi. Sequencing was carried out on ABI PRISM 3100 Genetic Analyzer. Sequencing results were analyzed through Chromas (v.2.6.6) software and BLAT (Ensembl genome browser).

Sequencing was performed on ABI PRISM 3100 Genetic Analyzer. Sequencing results were analyzed through Chromas (v.2.6.6) and BLAT (Ensembl genome browser 108) using the reference sequence (ENSCHIT00000041611.1).

RESULTS

The morphological features recorded in current study as hairy with compact body, big horns in the local breed (Fig. 1). Local breed (herds with a minimum size of 100 animals) are raised by the transhumant herders (Fig. 2) living in hilly/mountainous areas for meat production. Medium straight body, light hairy coat curved, and spiral horns observed in the mixed (cross) breeds (Fig. 3).

The lasi breed mostly originated from the Sindh province were observed as with straight body, higher birth weight, adult body weight, greater height and body length as compared to other two breeds (Table I) Hair less coat or hair observed mostly at hind legs. Ears were observed long (Fig. 3). Lasi goat breeds are raised mostly by agricultural farmers due to their high prolificacy and milk production.

Fig. 1. Local breed: herds with a minimum size of 100 animals

Fig. 2. Transhuman herders: living in hilly/mountainous areas

Fig. 3. Cross (Mixed) breed

During the study it was observed that in many parts, especially in hilly areas most of the herds were with heterogenous population (Fig. 4) of different breeds resulting the production of unintended crossed animal without any selection/breeding strategy.

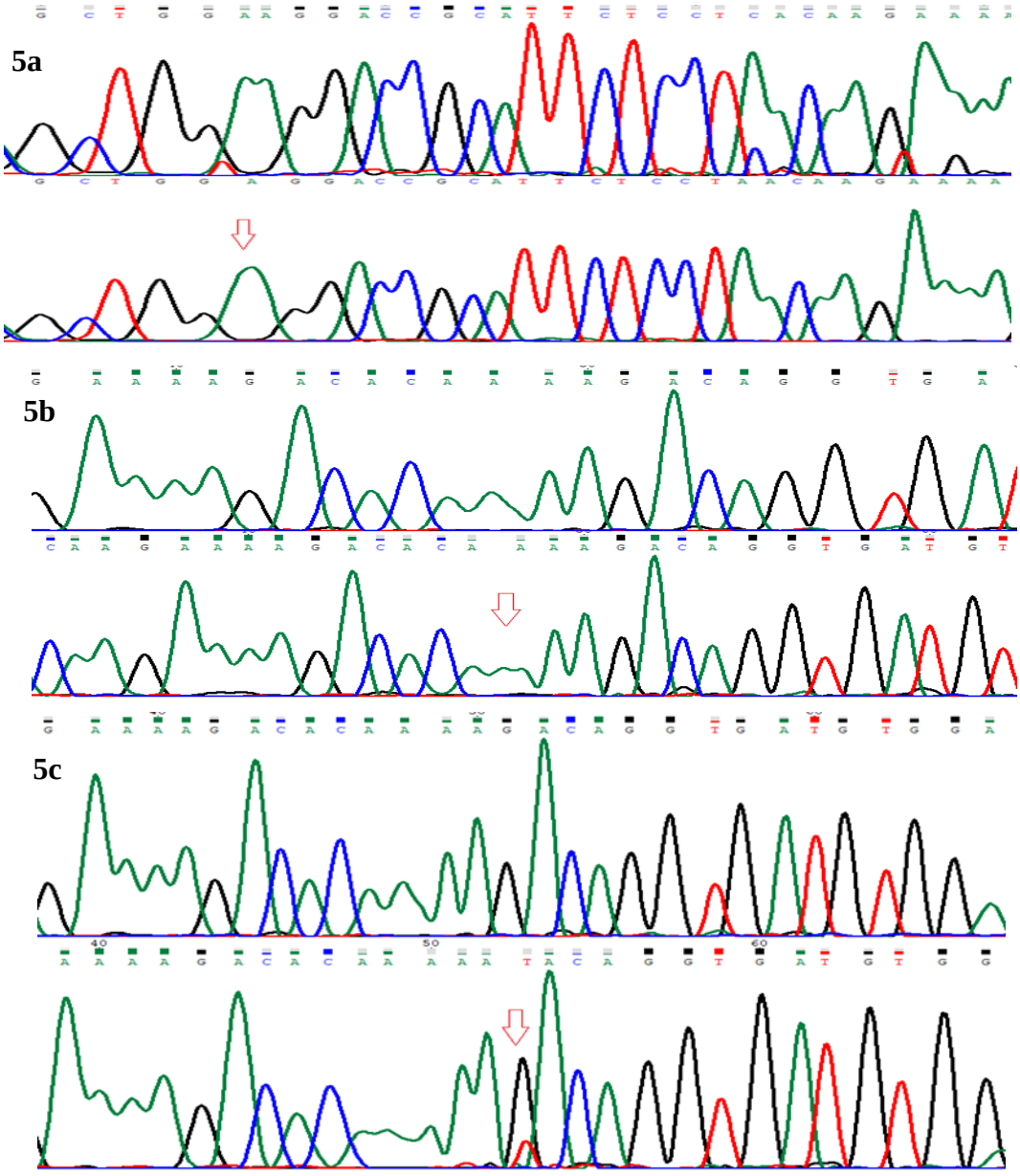
Fig. 3. Heterogenous herd: breeds resulting the production of unintended crossed animal

The litter size was reported in local and mixed breeds with single birth; less often twin births have also been reported in selective animals, whereas litter size in lasi breed reported with twins and sometimes

Table I. Phenotypic data (Average) recorded in current study

Phenotypic Characteristics	Breeds		
	Lasi	Local	Mixed
Birth Weight (kg)	2.5	2.4	2.4
Weaning Weight (kg)	14 kg	13 kg	14 kg
Live weight (adult)	50 kg	47 kg	48 kg
Height adult (Inches)	29.6′′	26.7′′	27.3′′
Body length (Inches)	23.8′′	22.6′′	23′′

with triplet pregnancies. As per the interview from the farmers and local meat consumers the tenderness and taste of the meat reported better in local breed followed by the cross breed as compared to with that of the lasi breed. We identified 5 single nucleotide polymorphisms including 2 frameshift variations c.290-91delA (Fig. 5a) identified in 70% local and 20% cross breed whereas c.322-25insA (Fig. 5b) at exon 5 was identified in 60 % of the local breed, one copy number variant including c.322-25insA and heterozygous missense variation c.326G>T(p.R109I) at exon 5 (Fig. 5c) in 60 % samples of lasi breed and 20% of mixed breed, and 2 splice site variations c.630+13G>A (Fig. 5d) in all breeds and c.699-701insG (Fig. 5e) at exon/intron 10 boundary identified in 53% in the local breed.



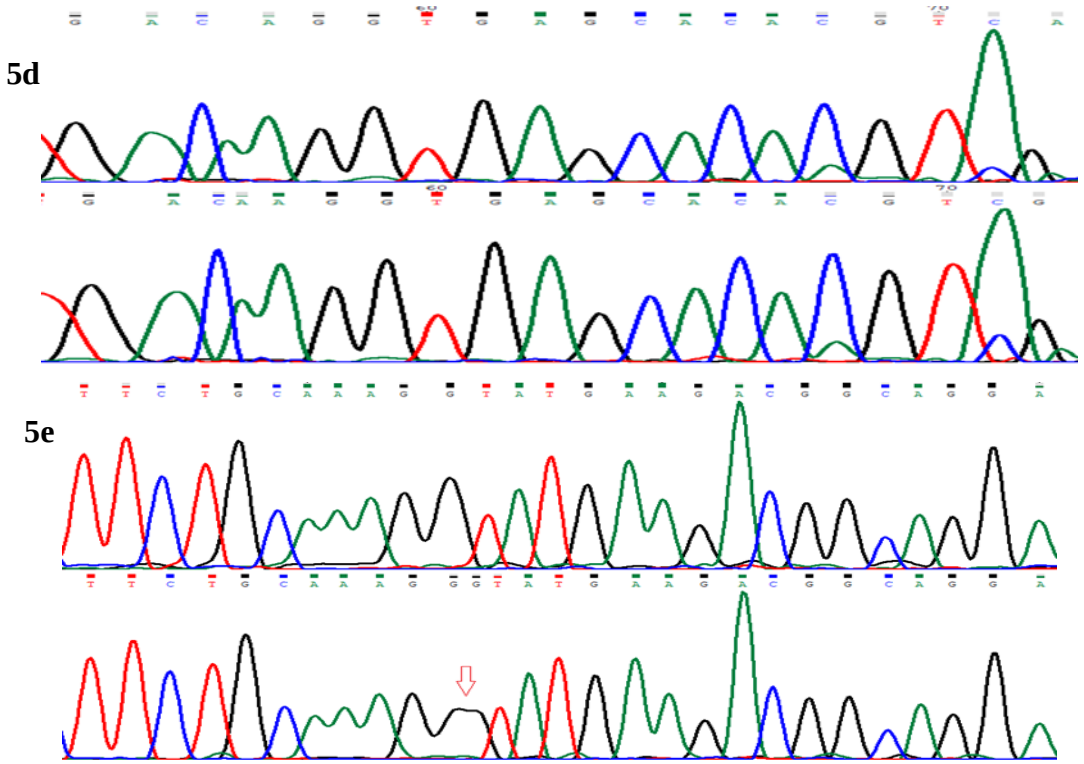


Fig. 5a. Frameshift mutation c.290-91delA at exon 5, **5b.** Frameshift variation c.322-25insA at exon 5, **5c.** Copy number variant c.322-25insA and c.326G>T(p.R109I), **5d.** c.630+13G>A, **5e. c**.699-701 insG at Exon/intron 10 (Splice site variation)

DISCUSSION

Tenderness is the most attributed quality that impacts meat consumption. Lot of factors including age, sex, breed, slaughtering conditions, nutritional, management and environmental factors influence the meat quality (21-23). *CAST* gene, a natural inhibitor of *μ*-calpain *(CAPN1)* gene, is one among the crucial factors influencing the meat quality and suggested to be adapted as a selection marker for meat quality traits (24). Studies suggest that variations identified in *CAST* gene in goat have been reported to influence the meat traits, this gene has also been reported to influence growth and development of animals (25-26) and tenderness of mutton and beef. (27-28). Genetic polymorphisms in *CAST* gene have also been reported by Othman *et al.,* (2016) to influence the meat quality in Egyptian sheep and goat breeds and suggested to be considered as a potential selection molecular marker.

Intronic variants identified in a study (29) suggest that the variant g.281G>A corelated with the cooking loss is associated with the meat quality of Indonesian goat breed Boerka. Studies carried out by to Goll *et al*. (2003), Lindholm-Perry *et al.* (2009), Saccà *et al.* (2019), Gracia *et al.,* (2020) indicate that the *CAST* gene variants are associated with shear force and tenderness of goat meat, mutton, and pork (19, 30-32). Phenotypic and genetic variants identified in current study are mostly breed specific and suggest that breeds that are being raised in different localities with different environmental conditions are required to be characterized phenotypically related to the productive traits. Even though a small sample size was used in the current study due funding limitations, the variants identified were extensive and give an indication that future studies could be carried out by using the *CAST* gene as a selective marker for characterization of the breeds based on their productive traits i.e. meat production as the molecular based markers are extensively being used to improve the product quality traits in farm animals.

Recent advancements in molecular genetics have further supported the association of *CAST* gene polymorphisms with economically important meat quality traits in small ruminants. High-throughput genotyping and sequencing technologies have enabled the identification of multiple SNPs within the *CAST* gene that are significantly associated with tenderness, drip loss, cooking loss, and shear force in goat and sheep breeds. For instance, SNP g.1797G>A within intron 5 of the CAST gene was significantly correlated with higher meat tenderness and lower shear force in Boer goats, suggesting its utility in selective breeding programs (33). Similarly, Saucedo-Uriarte *et al.,* (2023) demonstrated that specific *CAST* gene

polymorphisms in goat breeds like Sirohi and Barbari were closely associated with muscle fiber structure and postmortem proteolysis, impacting overall meat palatability (34). Furthermore, integration of *CAST* gene polymorphism data with phenotypic performance records through genomic selection models has shown promise in improving selection accuracy and genetic gain in meat production traits (35). Therefore, identification and validation of *CAST* gene variants in local Balochistani goat breeds could significantly enhance marker-assisted selection (MAS) strategies, contributing to sustainable meat production and genetic improvement in arid and semi-arid regions.

CONCLUSION

In the line to the studies discussed in the current manuscript the *CAST* gene variants identified in current study are also breed specific and may be either positively or negatively correlated with the meat traits within and among the breeds under study and suggest that the various small ruminant breeds raised in Balochistan may be characterized extensively following the standard phenotypic and genotypic characterizing procedures for the future selection of the breeds based on their adaptability and diverse productive traits.

Conflict of Interest:

We all authors submit and certify that there is no conflict of interest related to the study presented in this manuscript for publication.

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Authors' contribution:

AHB Conceptualization, Data curation & Writing; NB Project administration; MHK Formal analysis; MJ Methodology; NZH Investigation; KW Investigation; IBM Statical analysis and AS Critical review & editing.

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