

GENETIC POLYMORPHISM OF OVINE CALPAIN GENE IN BANDUR SHEEP

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Abstract: The present study was conducted to determine the polymorphism of calpain gene in Bandur sheep. The blood samples were collected from 100 Bandur ram lambs distributed over core area of the home tract, as well as from Livestock Research and Information Centre (S), KVAFSU, Nagamangala. Miller's high salt was employed for isolation of genomic DNA from venous blood. The quantity and quality of DNA was ascertained by spectrophotometer and 0.8 per cent agarose gel electrophoresis. A 192 bp of exons 5 and 6 including intervening intron of ovine calpain regulatory gene was amplified by PCR using published primers. The PCR-SSCP analysis revealed genotype frequency of 0.672 and 0.295 for AA and AB, and allele frequency of 0.820 and 0.180 for A and B, respectively. The observed and expected heterozygosities were 0.360 and 0.295, respectively. The PCR products corresponding to each genotype were got sequenced. Alignment of A and B allele of calpain by CLC Main Workbench 6.8.1. showed addition/ insertion of one nucleotide 'A' in B allele. The sequenced calpain segments was used as query and subjected to nucleotide blast at NCBI, which revealed 100, 99, 97, 97 and 89 per cent identity with *Ovisaries*, *Capra hircus*, *Bos taurus*, *Bubalusbubalis* and *Sus scrofa*. Since the Calpain gene is considered as candidate gene for growth and carcass/ meat quality traits, further study may be conducted to ascertain the association of these polymorphisms with the growth and carcass/ meat quality traits.

Keywords: Calpain, Bandur, Sheep, SSCP, Polymorphism.

INTRODUCTION

The sheep breeds of Karnataka are small to medium in size and are raised only for mutton purpose. Among all sheep breeds, Bandur sheep (another popular name of Mandya sheep) is considered as the best meat breed of the country as far as conformation is concerned (Acharya, 1982). Although it is highly rated for its meat quality/ taste, there is scant literature on the carcass and meat quality traits.

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The traits which are important for meat production are carcass and meat quality traits. They are influenced by the environment *viz.*, management conditions, nutritional status and pre and post slaughter handling, and they also have relatively moderate to high genetically controlled variation in the traits (Wheeler *et al.*, 2004). This indicates that a considerable proportion of the variation is under genetic control and hence could be improved by selection. Using genetic selection to improve carcass and meat quality traits is different from many traits that can be measured on live animals because direct measurements for these traits are not available on the animals to be used in future as breeding stock (Williams, 2008). In such cases, gene based methods have the potential to facilitate the improvement of traits.

The 'Calpain-Calpastatin System' (CCS) comprises a family of calcium dependent neutral proteases and found in most of the animal tissues. It influences many important processes including muscle development and degradation, regulation of protein degradation and rebuilding, cell cycle, organogenesis, post mortem meat tenderization, cataract formation and cell death (Merin *et al.*, 1998). The first protein of CCS recognized was m-calpain in the year 1976 (initially named as CAF) and after several years μ calpain and calpastatin were recognized. Calpain (*CAPN*) activity depends on Ca^{2+} , and proteolysis of myofibrils by calpain plays a significant role in muscle growth and tenderness of slaughtered meat (Sensky *et al.*, 2000). It is now accepted that Calpain mediated degradation of myofibrillar proteins is responsible for the postmortem meat tenderization, which occurs during storage at refrigeration temperatures (Chung *et al.*, 2002).

Since both μ and m calpain appear to be capable of cleaving the same target proteins, it is difficult to determine unequivocally, which is likely to be most important during the postmortem conditioning period. In general, the m isoform persists longer than the less stable μ calpain in ageing muscle from all species studied, including pig (Sensky *et al.*, 1996).

Skeletal muscle cells contain sufficient calpains to destroy all Z disks in these cells in 5 – 10 min. Hence, most of the calpain in muscle cells must be inactive most of the time. It seems likely that calpain activity is regulated by calpastatin levels and by the Ca^{2+} requirement of the calpains (Goll *et al.*, 1998).

The Calpain gene was investigated as a potential candidate gene for quantitative trait locus (QTL) affecting meat tenderness (Koochmaraie, 1992). Two *CAPN* alleles (A and B) from exons 5 and 6 (including intron between them) have been identified and are easily detected by PCR amplification and SSCP process (Chung *et al.*, 1999).

The present study was aimed to determine the polymorphism of calpain gene, in order to find effective alleles influencing carcass and meat quality traits in Bandur sheep.

MATERIALS AND METHODS

Blood Collection and Isolation of Genomic DNA: To determine the genetic variability in calpain gene, blood samples were collected from 100 Bandur ram lambs distributed over the villages of Malavalli taluk, Mandya district (core area of the home tract) as well as from Livestock Research and Information Centre (Sheep), KVAFSU, Nagamangala. High salt method as described by Miller *et al.* (1988) was employed for isolation of genomic DNA from venous blood. The purity and yield of genomic DNA were ascertained by spectrophotometer and 0.8 per cent agarose gel electrophoresis.

PCR amplification: Amplification of exons 5 and 6 and intervening intron (*CAPN* 456) of ovine m-Calpain regulatory gene was done employing published primers *viz.*, Forward: 5' AAC ATT CTC AAC AAA GTG GTG 3' and Reverse: 5' ACA TCC ATT ACA GCC ACC AT 3'(Shahroudi *et al.*, 2006; Dehnavi *et al.*, 2012). The amplification was done in a total volume of 25 μ l consisted of 12.5 μ l of Red PCR master mix, 1 μ l (10 pmol/ μ l) each of forward and reverse primer, 9.5 μ l of PCR grade water and 1 μ l (100 ng) of template DNA. The PCR reaction was carried out with an initial denaturation temperature of 95 °C (3 min), 35 cycles of 94 °C (45 sec), 59 °C (1 min) and 72 °C (90 sec), followed by final extension at 72 °C (10 min). The PCR amplified products were confirmed by resolving on 1.5 per cent agarose in parallel with 100 bp DNA ladder. Gel electrophoresis was carried out at a constant voltage of 100 V for 60 min in 1X TAE buffer.

Genotyping by PCR-SSCP: The PCR products were mixed with denaturing formamide dye consisting of formamide (95 %), xylene cyanol (0.025%), bromophenol blue (0.025%) and 0.5 M EDTA. The mixture was denatured at 95 °C for 5 minutes and snap cooled on ice for 3 minutes. Polymorphism of calpain gene was determined by using 12 per cent polyacrylamide gel (50:1; Acrylamide: Bisacrylamide). The electrophoresis was carried out at 100 V for 3 hours and the resolved bands were visualized after silver staining.

DNA sequencing and Statistical Analysis: Based on visualization of different band patterns, genotypes were determined. The allele number, allele frequency, genotype frequency, and observed and expected heterozygosity were calculated.

The PCR products corresponding to different patterns were custom sequenced using primers used for amplification. The sequencing was done at Chromous Biotech private limited, Bangalore and the resultant sequences were analyzed by CLC Main Workbench software

(CLC BIO, 2011). The PCR amplified sequence of *CAPN* gene was used as query and subjected to nucleotide blast at NCBI (<http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi>) for sequence homology searches in public databases.

RESULTS AND DISCUSSION

A 192 bp of exons 5 and 6 including intervening intron of ovine calpain regulatory gene was amplified by PCR (Figure 1). The PCR-SSCP analysis of PCR products revealed two patterns *viz.*, AA and AB with frequency of 0.672 and 0.295, respectively (Figure 2). The allele frequency was 0.820 and 0.180 for A and B, respectively. The observed and expected heterozygosities were 0.360 and 0.295, respectively. The allele and genotype frequencies and observed and expected heterozygosities for *CAPN* gene are presented in Table 1.

The occurrence of two genotypes AA and AB in the present study confirm the earlier reports of Shahroudi *et al.* (2006), Nassiry *et al.* (2007) and Dehnavi *et al.* (2012) in Iranian Karakul sheep, Kurdi sheep and Zel sheep. The frequencies of AA and AB reported by these workers were 0.70 and 0.30, 0.92 and 0.08, and 0.69 and 0.31, for the above mentioned breeds, respectively, whereas the respective allele frequencies were 0.85 and 0.15, 0.96 and 0.04, and 0.845 and 0.151 for A and B. The observed and expected heterozygosities were 0.2963 and 0.254 in Iranian Karakul sheep (Shahroudi *et al.*, 2006), and 0.0824 and 0.0794 in Kurdi sheep (Nassiry *et al.*, 2007), respectively.

In contrast to the present study, Azari *et al.* (2012) observed 3 different patterns (G1, G2 and G3) with the genotypic frequency of 0.082, 0.892 and 0.027, respectively in Dalagh sheep of Iran and the results were in accordance with the earlier reports of Tahmoospour (2005), who reported three genotypes (AA, AB and BB) with allele frequency of 0.56 and 0.44 for A and B alleles, respectively in Baluchi sheep.

Arora *et al.* (2014) found allele frequency of 0.603 and 0.397 for A and B alleles, respectively and observed genotype frequency of 0.388, 0.429 and 0.183 per cent for AA, AB and BB genotypes, respectively. Further the observed and expected heterozygosities were 43.5 and 56.9 per cent, respectively in 11 Indian sheep breeds. These results were in contrast to those obtained in the present study.

Overall, all the earlier workers have shown a higher frequency of allele A in the different sheep breeds study, which is confirmed in the present study.

The selected PCR products corresponding to two genotypes: AA and AB were got sequenced and the expected size of 192 bp fragment was confirmed.

The alignment of A and B allele of *CAPN* by CLC Main Workbench 6.8.1. (Figure 3) showed addition of one nucleotide 'A' in B allele at 89 bp position (Figure 4). Arora *et al.* (2014) identified SNP g.44C > T in *CAPN4* gene of Indian sheep breeds. Zhou *et al.* (2007) reported presence of three SNPs in exon 10 region of Ovine *CAPN* 3.

The PCR amplified sequence of *CAPN* gene (192 bp) was used as query and subjected to nucleotide blast at NCBI, which revealed 100, 99, 97, 97 and 89 per cent identity with *Ovisaries* ([AF309634.1](#)), *Capra hircus* ([AY935995.1](#)), *Bos taurus* ([EF139087.1](#)), *Bubalusbubalis* ([XM_006068758.1](#)) and *Sus scrofa* ([AJ410870.2](#)), respectively (Table 2).

The aim of the present study was to determine the polymorphism of calpain gene in Bandur sheep. The results confirmed that the PCR-SSCP is an appropriate tool for determining genetic variability in calpain locus. Since calpain gene is considered as candidate gene for growth and carcass/ meat quality traits, further study may be conducted to ascertain the association of these genotypes with the growth and carcass/ meat quality traits.

REFERENCES

- [1] Acharya R M. 1982. Sheep and Goat breeds of India. Food and Agriculture Organization of the United Nations.
- [2] Arora R R, Yadav H S and Yadav D K. 2014. Identification of novel single nucleotide polymorphisms in candidate genes for mutton quality in Indian sheep. *Animal Molecular Breeding* 4(1): 1-5.
- [3] Azari M A, Dehnavi E, Yousefi S and Shahmohamdi L. 2012. Polymorphism of Calpastatin, Calpain and Myostatin genes in native Dalagh sheep in Iran. *Slovak Journal of Animal Science* 45(1): 1-6.
- [4] Chung H Y, Davis M E and Hines H C. 1999. A DNA polymorphism of the bovine calpastatin gene detected by SSCP analysis. *Animal Genetics* 30: 66.
- [5] Chung H Y, Kim C D, Cho C Y, Yeon S H, Jin H J, Joen K J, Kim H C, Lee H J, Hines H C and Davis ME. 2002. Effect of calpastatin gene on growth and carcass traits of Korean native cattle. *Proceedings of the 7th World Congress Applied to Livestock Production*. August 2002, Montpellier, France.
- [6] Dehnavi E, Azari M A, Hasani S, Nassiry M R, Mohajer M, Khan Ahmadi AR, Shahmohamadi L and Yousefi S. 2012. Association between yearling weight and calpastatin and calpain loci polymorphism in Iranian Zel sheep. *Iranian Journal of Applied Animal Science* 2(2): 131-135.

- [7] Goll D E, Thompson V F, Taylor R G and Ouali A. 1998. The calpain system and skeletal muscle growth. *Canadian Journal of Animal Science* 78: 503- 512.
- [8] Koohmaraie M. 1992. The role of the Ca²⁺ dependent protease (Calpains) in post-mortem proteolysis and meat tenderness. *Biochimie*74: 239-245.
- [9] Merin L B, Kendal T V, Thompson F and Goll D E. 1998. Change in the Calpains and Calpastatin during Post Mortem Storage of bovine Muscle. *Journal of Animal Science* 76: 2415-2434.
- [10] Miller S A, Dykes D D and Polesky H F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Research* 16: 1215.
- [11] Nassiry M R, Shahroudi F E, Tahmoorespour M and Javadmanesh A. 2007. Genetic Variability and Population Structure in Beta-lactoglobulin, Calpastatin and Calpain Loci in Iranian Kurdi Sheep. *Pakistan Journal of Biological Sciences* 10(7): 1062-1067.
- [12] Sensky P L, Parr T, Bardsley R G and Buttery P J. 1996. The relationship between plasma epinephrine concentration and the activity of the calpain enzyme system in porcine longissimus muscle. *Journal of Animal Science* 74: 380–387.
- [13] Sensky P L, Parr T, Bardsley R G and Buttery P J. 2000. Postmortem proteolysis in muscle and meat quality and phenotypic and genetic correlations for bovine post rigor calpastatin activity, intramuscular fat content, variable activity of the calpain proteolytic system. Meat and Livestock Commission, Loughborough, UK.
- [14] Shahroudi F E, Nassiry M R, Valizadh R, Moussavi A H, Tahmoorespour M and Ghiasi H. 2006. Genetic polymorphism at *MTNR1A*, *CAST* and *CAPN* loci in Iranian Karakul sheep. *Iranian Journal of Biotechnology* 4(2): 117-122.
- [15] Tahmoorespour M. 2005. Study on the calpain and calpastatin gene polymorphisms by polymerase chain reaction–single strand conformation polymorphism and its relation to average daily gain in Iranian Baluchi sheep. Ph.D., Thesis, Ferdowsi University, Mashhad, Iran.
- [16] Wheeler T L, Cundiff L V, Shackelford S D and Koohmaraie M. 2004. Characterization of biological types of cattle (Cycle VI): Carcass, Yield and *longissimus dorsi* palatability traits. *Journal of Animal Science* 82: 1177-1189.
- [17] Williams J L. 2008. Genetic control of meat quality traits. *Meat Biotechnology* 21-60.
- [18] Zhou H, Hickford J G and Gong H. 2007. Polymorphism of the ovine calpastatin gene. *Molecular and Cellular Probes* 21: 242-244.

Table 1: Allele and genotypic frequencies and observed and expected heterozygosity for CAPN genotype

No. of Samples	Allele frequency		Genotypic frequency			Observed Heterozygosity	Expected Heterozygosity
	A	B	AA	AB	BB	Ho	He
100	0.820	0.180	0.672	0.295	-	0.360	0.295

Table 2: Results of BLASTn showing the percentage of identity of the Exons 5 and 6 and intervening intron region of the Ovine CAPN gene sequence

Accession. No	Description	Max Score	Total Score	Query coverage (%)	Identity (%)
<u>AF309634.1</u>	<i>Ovisaries calpain 4 gene partial cds</i>	351	351	98	100
<u>AY935995.1</u>	<i>Capra hircus calpain 4-like gene, complete sequence</i>	287	287	83	99
<u>EF139087.1</u>	<i>Bos taurus calpain small subunit 1 (CAPN 1S) gene, complete cds</i>	327	327	100	97
<u>XM_006068758.1</u>	PREDICTED <i>Bubalus bubalis calpain, small subunit 1 (CAPNS1), mRNA</i>	110	162	48	97
<u>AJ410870.2</u>	<i>Sus scrofa COX7A1 gene, CAPNS1 gene, CKAP1 gene, POLR2I gene and CLIPR-59 gene (partial)</i>	233	233	100	89

FIGURES

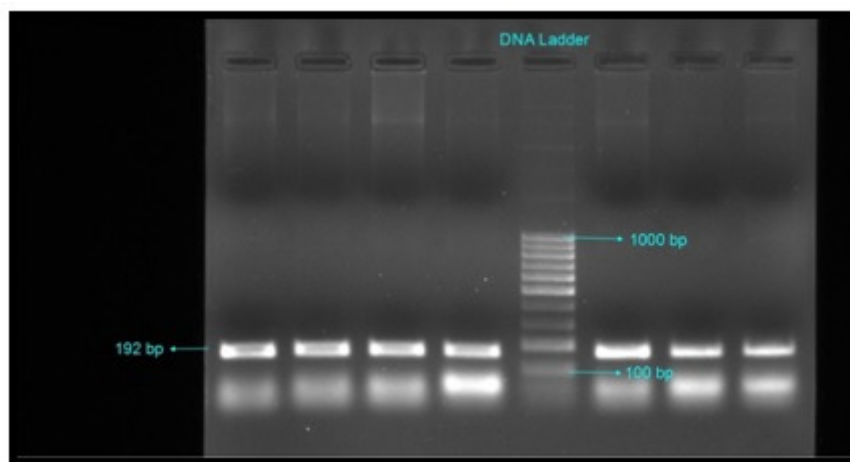


Figure 1: Agarose electrophoresis gel (1.5 %) showing amplification of Calpain gene (Exon 5 and 6 and the intervening intron)

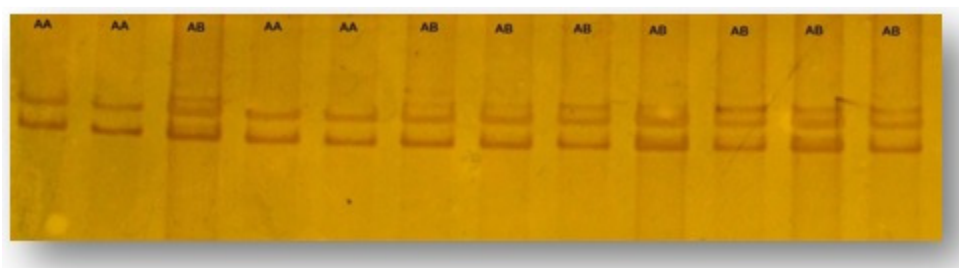


Figure2: Poly Acrylamide Gel Electrophoresis (12%) showing SSCP patterns of Exons 5 and 6 and intervening intron of Calpain gene



Figure 3: Alignment of A and B allele sequences of Calpain by CLC Main Workbench 6.8.1.

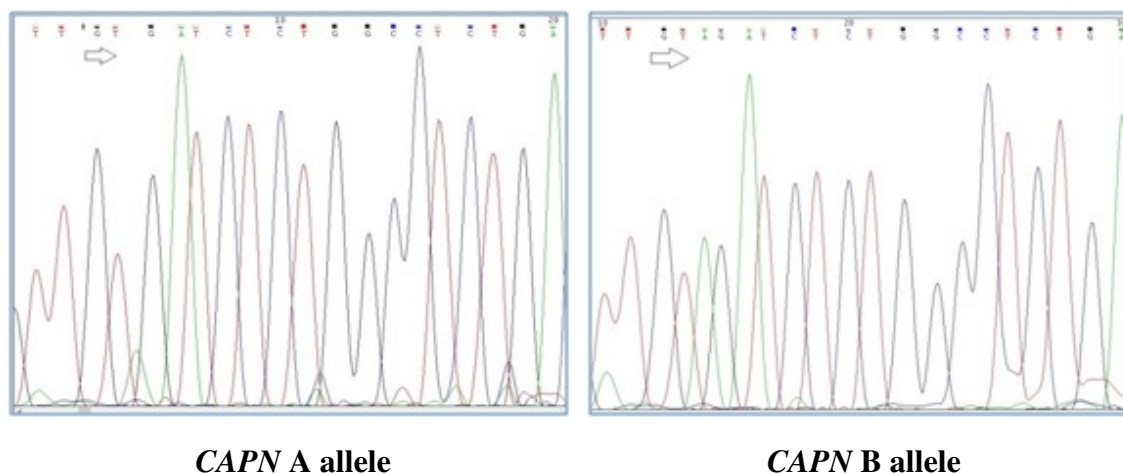


Figure 4: Chromatograms showing addition of 'A' nucleotide at 89 bp position