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Genetic Polymorphism of *CAPN1* Gene in Sirohi Goat

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ABSTRACT

The *CAPN1* gene plays an important role in post mortem tenderization of meat and it is a main candidate gene for assessing the meat quality characteristics of beef. In this study, 59 nucleotide variations in different regions (exon 3, exon 4 and exon 11 and its flanking region) of *CAPN1* gene were compared with *Bos taurus*. The fragment of exon 3 and their flanking region were sequenced and thirteen nucleotide changes in this region were identified. Ten variations and one deletion (1861{C}) were observed in the fragment (480 bp) of exon 4 and its up and downstream regions. On sequencing of exon 11 and its flanking region thirty variations and eight deletions at the position 175-182 (TCCTTCTG), one addition at the position 182-183 (G) were observed. The variations observed in this study can be used in further research for association between gene polymorphisms and goat meat quality traits.

Key words: *CAPN1* gene, genetic polymorphism, Sirohi goat

INTRODUCTION

Goats are uniformly distributed across the climatic regions of India from cold desert of Ladakh in north to saline water of Andaman in south and from dry desert of Rajasthan in west to humid climate of Bengal in eastern regions of India (Singh *et al.*, 2008). The genetic improvement of beef cattle has been sought as a means to improve production efficiency for many decades. These efforts have been successful in traits that comprise the recording and evaluation programs (Miller, 2010).

There is a considerable interest in the application of molecular genetic technologies in the form of specific DNA markers that are associated with various QTL to promote more efficient and relatively easy selection and breeding of farm animals with an advantage for the inheritable traits of growth rate, body weight, carcass merit, feed intake as well as milk yield and composition (Spelman and Bovenhuis, 1998; Othman *et al.*, 2011).

The *CAPN1* gene, mapped to the telomeric end of the BTA29 linkage group (Smith *et al.*, 2000), encodes the protease μ-calpain, which degrades myofibrillar proteins post-mortem (Allais *et al.*, 2011). Two enzymes responsible for beef tenderness are the micromolar calcium-activated neutral protease μ-calpain (CAPN1), which is encoded by the *CAPN1* gene and its inhibitor, calpastatin (CAST), which is encoded by the CAST gene (Koohmaraie *et al.*, 1996). The calpain/calpastatin system is an endogenous, calcium dependent proteinase system, theorized to mediate the proteolysis of the key myofibrillar proteins during post-mortem storage of carcass and cuts of meat at refrigerated temperatures (Koohmaraie *et al.*, 1995). Some markers in the *CAPN1* gene have been suggested to fill this role; the Amino Acid (AA) polymorphisms at position 316 (glycine/alanine) and 530 (valine/isoleucine) are the important markers associated with tenderness in many families or crossbred *Bos taurus* (Page *et al.*, 2002; Xin *et al.*, 2011).

As a first attempt to observe the *CAPN1* gene polymorphism in Indian goat breeds, the present study has been designed to analyze the genetic variation in exon 3, exon 4 and exon 11 along with their flanking regions of *CAPN1* gene in Sirohi goats.

MATERIALS AND METHODS

Animal, sample collection and DNA isolation: Twelve blood samples (5-6 mL) were obtained from jugular vein puncture in vacutainer tubes (BD Bioscience, Germany) pretreated with 0.25% Ethylene Di Amine Tetra Acetic acid (EDTA). The genomic DNA for PCR amplification was isolated from white blood cells using the standard phenol-chloroform extraction method of Sambrook *et al.* (1989) with minor modification. After checking the quality and quantity, DNA was diluted to final concentration of 100 ng μL^{-1} in nuclease free water and stored at 4°C.

Primer designing and PCR amplification: Based on cattle *CAPN1* gene sequence (AF 252504S1 and AF 252504S2), three primer sets were designed to amplify complete exon 3, exon 4 and exon 11 along with intervening introns of *CAPN1* gene. Primers were designed by LASERGENE software (DNASTAR, version 4.0, Inc., USA). Sequences of designed primers are given in Table 1. PCR amplifications were performed in a 25 µL reaction volume with approximately 100 ng genomic DNA, 1 U *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India), 10×buffer, 1.5 mM $MgCl_2$, 200 µM each dNTP and 10 pM of each primer. PCR was carried out in a Bio-Rad iCycler (Germany). PCR conditions were as follows: 2 min at 95°C, 30 cycles of 94°C for 30 sec, specific annealing temperature for 30 sec and 72°C for 30 sec, with final extension at 72°C at 10 min. Amplified product were checked on 1.6% agarose gel (w/v) in 1×TAE buffer.

Sequence analysis: Twelve PCR products were purified with the NP PCR purification kit (Taurus Scientific, USA) and sequenced by ABI 3100 (Applied Biosystem, USA). The BLAST algorithm was used to search the NCBI GenBank (http://www.ncbi.nlm.nih.gov/) databases for homologous sequences. Sequence variants were verified by chromatograms. The sequence analysis was carried out using various modules of DNASTAR version 4.0, Inc., USA, for protein translation-EDITSEQ sequence alignment and contigs comparison-MEGALIGN.

Table 1: Primers and PCR analysis parameters

Location	Primer	Primer sequence (5'-3')	Base pair (bp)	Product length (bp)	Annealing temp. (°C)
Exon 3	CAPEX 3	F: CTGAGTGAGGAGTGGGAATAGGAC	24	402	60.0
		R: ATCTTTGCCCAGTGAGTGCCATAC	24		
Exon 4	CAPEX 4	F: AGTCCCAGGTTCTCCCCAGTA	21	480	56.9
		R: CTTTCTTTTCTCCAGTGTGC	20		
Exon 11	CAPEX 11	F: TTCAGGTCTCAGCTCACAGGTCAC	24	637	59.9
		R: CTCCCCAAGGTCAGGTTTAGG	21		

RESULTS

The fragment of exon 3 (70 bp) and its flanking region (402 bp) are sequenced and sequence analysis revealed thirteen nucleotide substitutions and four deletions in Sirohi goat. Out of thirteen nucleotide substitutions, only one non-synonymous substitution was observed in exon 3 at the position 1601 G→A (Transition). The deletion was observed at the position 1493 T. In partial intron 2 one deletion (1493 {T}); two transitions at the 1501 C→T; 1545 C→T and one transversion 1480 G→T were observed. In partial intron 3 seven transitions at the position 1640 C→T, 1689 C→T, 1709 G→A, 1724 C→T, 1788, C→T, 1795 T→C and 1797 C→T, two transversion 1654 G→C and 1817 C→A and three deletions position 1661 A, 1662 G and 1663 T were observed (Table 2).

The 480 bp fragment of exon 4 (119 bp) and their flanking region were sequenced. The sequence analysis of the amplified samples revealed nucleotide substitutions within intron 3, exon 4 and intron 4 regions. A total ten variations and one deletion were identified in caprine *CAPN1* gene as compared with *Bos taurus* (AF252504S1). Out of the ten variations four were in coding regions and remaining were in the intronic regions and the deletion was observed at the position 1861 (C) in partial intron 3. Out of the four variations found in coding region, three non-synonymous substitutions, two transitions and one transversion were observed at the position 1895 C→T, 1931 C→A and 1982 T→G. The substitutions 1895 C→T, 1931 C→A and 1982 T→G resulted in change of amino acid serine (polar) to phenyl alanine (non polar), non polar proline to polar histidine and non polar leucine to polar arginine, respectively (Table 3).

Comparative sequence analysis of the fragment covering intron 10-exon 11-intron 11 region of *CAPN1* gene in Sirohi breed of domestic goat was identified with thirty variations and eight deletions (at the position 175-182 {TCCTTCTG}) one addition (at the position 182 183 {G}). Five variations were detected in coding region of exon 11 and remaining twenty five variations were found in intronic region. Three variations of exon 11 are non-synonymous in nature. The variation 410 G→A, 485 G→A and 521 C→T resulted amino acid substitutions from arginine (polar) to glutamine (polar), glycine (polar) to glutamine(polar) and alanine (nonpolar) to valine (nonpolar), respectively. Two variations 453 C→T and 494 T→A are synonymous in nature and they do not affect the protein product of the gene (Table 4).

Table 2: Nucleotide variations of exon 3 and their flanking region in *CAPN1* gene of Indian goat

Region	Nucleotide position	Exotic cattle (AF252504S1)	Variation	Polarity	Amino acid change	Type of change
Intron 2	1480	G	T		Non coding	Transversion
	1493	T	-			Deletion
	1501	C	T		Non coding	Transition
	1545	C	T		Non coding	Transition
Exon 3	1601	G	A	P→P	Thr→Thr	Transition
Intron 3	1640	C	T		Non coding	Transition
	1654	G	C		Non coding	Transversion
	1661	A	-			Deletion
	1662	G	-			Deletion
	1663	T	-			Deletion
	1689	C	T		Non coding	Transition
	1709	G	A		Non coding	Transition
	1724	C	T		Non coding	Transition
	1788	C	T		Non coding	Transition
	1795	T	C		Non coding	Transition
	1797	C	T		Non coding	Transition
	1817	C	A		Non coding	Transversion

Thr: Threonine, -: Deletion, P: Polar

Table 3: Nucleotide variations of exon 4 and their flanking region in *CAPN1* gene of Indian goat

Region	Nucleotide position	Exotic cattle (AF252504S1)	Variation	Polarity	Amino acid change	Type of change
	1856	C	T		Non coding	Transition
Intron 3	1861	C	-			Deletion
	1862	G	A		Non coding	Transition
	1863	G	T		Non coding	Transversion
Exon 4	1895	C	T	P→NP	Ser→Phe	Transition
	1931	C	A	NP→P	Pro→His	Transversion
	1982	T	G	NP→P	Leu→Arg	Transversion
	2019	A	G		Non coding	Transversion
Intron 4	2030	G	A		Non coding	Transversion
	2034	T	G		Non coding	Transversion
	2039	C	T		Non coding	Transition

-: Deletion, NP: Nonpolar, P: Polar, Ser: Serine, Phe: Phenyl alanine, Pro: Proline His: Histidine, Leu: Leucine, Arg: Arginine

Table 4: Nucleotide variations of exon 11 and flanking region in *CAPN1* gene of Sirohi goat

Region	Nucleotide position	Exotic cattle (AF252504S2)	Sirohi goat	Polarity	Amino acid change	Type of change
	127	C	T			Transition
	137	C	T			Transition
	147	G	C			Transversion
	148	C	A			Transversion
	152	A	G			Transition
	175	T	-			Deletion
Intron 10	176	C	-			Deletion
	177	C	-			Deletion
	178	T	-			Deletion
	179	T	-			Deletion
	180	C	-			Deletion
	181	T	-			Deletion
	182	G	-			Deletion
			G			Addition
	211	C	T			Transition
	234	C	T			Transition
	236	A	C			Transversion
	328	T	C			Transition
	254	C	A			Transversion
	258	T	C			Transition
	261	T	C			Transition
	263	C	T			Transition
	282	G	C			Transversion
	317	C	T			Transition
	325	T	C			Transition
	326	G	T			Transversion
Exon 11	410	G	A	P→P	Arg→Gln	Transition
	453	C	T	P→P	Ser→Ser	Transition
	485	G	A	P→P	Gly→Gln	Transition
	494	T	A	NP→NP	Ala→Ala	Transversion
	521	C	T	NP→NP	Ala→Val	Transition
Intron 11	547	A	G			Transition

Table 4: Continue

Region	Nucleotide position	Exotic cattle AF252504S2	Sirohi goat	Polarity	Amino acid change	Type of change
	551	T	C			Transition
	560	T	C			Transition
	563	A	G			Transition
	586	C	G			Transversion
	592	T	C			Transition
	602	T	C			Transition
	608	G	T			Transversion

NP: Non polar, P: Polar, Ala: Alanine, Gly: Glycine, Gln: Glutamine, Ser: Serine, Arg: Arginine, -: Deletion

The sequences for exon 3, exon 4 and exon 11 were deposited in GenBank database with accession numbers HQ916357 and GU784847, respectively.

DISCUSSION

SNP/variations analysis is a well-established tool for the identification of genes associated with traits of economic importance in livestock populations. More recently, haplotype analysis has become an area of intense research for complex genetic phenotypes (Stone *et al.*, 2005). Exonic DNA variants of genes contain the genetic information that is transcribed and translated to the protein product that produces, directly or indirectly, observed phenotypes of traits. Sequence variations, the bases of genetic diversity and evolution, in the exons may affect positively or negatively the function of protein products and hence, production traits (Ibeagha-Awemu *et al.*, 2008). Further the utilization of variation in the animal genome is always bound to naturally occurring mutations resulting in different individuals in a population. This phenomenon is called polymorphism. Many such mutations are silent and are identified as synonymous mutations. However, some polymorphisms allow changes in gene expression. When the polymorphic genes express those changes in protein levels, then it is called non-synonymous polymorphism. Although, some changes are deleterious, some are advantageous for production, reproduction disease resistance etc.

The *CAPN1* gene is known to play a key role in the post-mortem tenderization of meat (Casas *et al.*, 2006; White *et al.*, 2005; Schenkel *et al.*, 2006; Page *et al.*, 2004). G316A in exon 9 of the *CAPN1* gene, which resulted a substitution of alanine for glycine at position 316 of the CAPN1 protein one potential SNP marker, has a reported effect on meat tenderness (Cheong *et al.*, 2008). A new InDel marker, which segregates differently between cattle breeds and does not provide redundant information to that already provided by the other CAPN1 markers, was found in *CAPN1* gene (Iglesias *et al.*, 2011). These findings, plus the fact that CAPN1 markers in *Capra hircus* are scarce, encourage further investigations to test usefulness of SNPs identified in present study as meat quality markers and as a valuable marker for diversity studies in goats.

CONCLUSIONS

This is the first study on genetic variation in *CAPN1* gene of indigenous goat. The present investigation resulted in the identification of genetic variation in the *CAPN1* gene between goat and cattle, which might explain a portion of the variation of meat quality and tenderness in goat population. Considering the economic importance of meat quality trait to the livestock industry, *CAPN1* gene polymorphisms described may be useful tool much rigorous future studies of association between *CAPN1* genotype and performance, in Sirohi goat breeds.

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