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Identification of SNPs of CAST gene in three different cattle breeds of Pakistan

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Abstract

Calpastatin is the variable component of calpain system, encoded by CAST gene. In skeletal muscle growth and post mortem tenderness of meat there is an essential role of calpastatin. Cast gene is located on bovine chromosome 7. Its main purpose is to change the proteolytic action of enzymes reliable for post-mortem myofibril deterioration. Present study was aimed at identification of novel SNPs of Calpastatin gene in three different cattle breeds of Pakistan. In this study PCR-Gel Electrophoresis and DNA sequencing techniques were used for obtaining the results. In total 7 mutations were identified with the help of PCR-Gel Electrophoresis and DNA Sequencing technique. Based on Genetic code, we have identified (02) mutations in Exon-5 of Red Sindhi cattle breed including a silent mutation at 80 bp in which CAA(Glutamine) codon was changed to CAG(Glutamine) but does not causes any change of Amino acid hence will not effect on meat qualities of the Red Sindhi cattle, Another mutation was also found in Exon 5 of the Red Sindhi cattle breed, which causes change of codon from TGT to CGT, in which former was coding for the Cystine and later is coding for Arginine Amino acid change from non-essential to essential. In Sahiwali cattle, 02 mutations were also identified in exon 3, in which AAA codon was changed to ACA at 27 bp, which results in substitution of Lysine to Threonine amino acid. Second mutation caused the change of codon from ATC to AGC which results in change of amino acid from Isoleucine to serine amino acid. We have also identified 03 mutations in Exon5 of Tharparker cattle breed. First one was identified at 35 bp of Exon 5, in which GTA codon was changed to GTG, which does not cause any change of amino acid so called silent mutation (Valine to Valine). Second mutation was identified at 57 bp in which GAA codon was changed into AAA, which results in change of Glutamine amino acid to Lysine Amino acid. Third mutation resulted in change of TAT codon to TGT codon that caused change of Thyronine amino acid to Cysteine amino acid. Based on this study we suggest that Exon 5. Essential mutation can be effect the meat properties in position manner and hence can be used in Marker Assisted selection of this cattle breed.

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Introduction

One of the most important priorities of the beef industries is to enhance the meat quality and tenderness of meat is the crucial characteristic for consumer's acceptance (Morgan *et al*., 1991). It is need to resolve the problem of manufacturing erratically tender meat. Much information on the inheritance of the meat tenderness trait has been published to determine the difficulty of variability in tenderness of meat, such as calpastatin action (Marshall, 1994; Bertrand *et al*., 2001; Riley *et al*., 2003). Calpastatin is a key segment of the CCs and skeletal muscle. calpastatin action is colossally identified with charge of muscle protein turnover and after death tenderization. Its miles communicated within all tissues communicating with calpains and also in skeletal muscle at a larger amount of movement than calpains. Even though minimum 8 calpain genes had been diagnosed, it is supposed that single calpastatin gene is most effective.

It is expected that calpastatin gene is of one hundred kb size consisting of 4 exons, and is positioned at 5q15 constituency on fifth chromosome in sheep. The calpastatin action correlate distinctly by way of increase in muscle rate, involving that skeletal muscle probable to effect from decreased protein deprivation because of concessional pastime of calpains or considerable growth in the activity of calpastatin (Goll *et al*., 1998 and 2003). Calpastatin is endogenous inhibitor protein which particularly acts upon calpains.

The calpin activity is considered to overcome through free calcium concentration as well as by the occurrence of calpastatin inhibitor. Initially Calpastatin was recognized while there was no calpain action might be observed in unsophisticated skeletal muscle of porcine homogenates until after precipitation at the pH 6.2 of the calpain (Dayton *et al*., 1976). Assay of the pH 6.2 supernatant showed that the trypsin-labile and heat-resistant factor were responsible for inhibition of calpain activity. It was complicated to evaluate and transmit firm calpastatin isoforms to its molecular weight on SDS PAGE. The first Calpastatin was purified from human's erythrocytes (Takano and Murachi, 1982). Consequent information illustrate purification from humans liver of a 107-kDa polypeptide that repressed 5 calpains per molecule (Imajoh *et al.,* 1984); isolation from bovine heart of a 145-kDa inhibitor (Mellgren and Carr, 1983); purification of a 210-kDa calpain inhibitor from rabbit skeletal muscle (Nakamura *et al.,* 1985); isolation of 172-kDa inhibitor from porcine skeletal muscle that repressed about 8 molecules of calpain per 172 -kDa molecule (Lepley *et al*., 1985); isolation from bovine brain of a 125-kDa inhibitor (Mohan and Nixon, 1995). Two size of calpastatin 68-kDa and 107-kDa were recognized from pig erythrocytes and pig heart muscle that shows distinction between tissues of similar animal (Takano *et al.,* 1988). So, the isolation of calpastatin from the various resources showed the heterogeneity of the inhibitor. Calpastatin is an intracellular polypeptide of 110 kDa having an N-terminal leader domain along with 4 repeating inhibitory domin containing three highly conserved areas called A, B, and C (Melloni *et al.,* 2006) each of them able to inhibit one calpain molecule distinctively (Maki *et al.,* 1987, Goll *et al*., 2003, Hanna *et al*., 2008). For bovine, the CAST gene was to be found and mapped in chromosome 7(Bishop *et al*., 1993) with relative position 117.8 cM by means of RFLP (Kappes *et al*.,1997). From sequencing the bovine calpastatin was recognized as having 35 exons in 130kb of sequence (Raynaud *et al*., 2005).

The genetical and physiological role of Calpastatin gene in meat tenderness in various animals has been accounted (Huff Lonergan *et al*., 1996; Boehm *et al*., 1998). Resultantly, two major mechanisms are associated to Calpastatin activity: 1st turnover diminution (amalgamation and decay) of muscle and escalating skeletal muscle growth. 2nd, diminishing Calpain action of muscle after carnage thereby decreasing tenderness of meat and superiority (Goll *et al*., 1998; Wheeler and Koohmaraei, 1999).

The intend of this study was to identify CAST gene polymorphisms and evaluate their genotypic effects in

03 different cattle breeds of Pakistan within Red sindhi Cattle Breed, Sahiwal Cattle Breed and Tharparkar Cattle Breed.

Materials and methods

In this study, total, 30 blood samples were taken from three different cattle breeds. 5ml of Blood were gathered from jugular vein of each chosen dairy animals into glass tube containing Anticoagulant (0.5 M EDTA). For ensuing examination blood were put at 4 °C. Genomic DNA was disengaged from entire blood by utilizing Thermo Scientific Genomic DNA decontamination pack from gathered blood samples. Primers designed for PCR amplification are shown in Table 1.

PCR amplification

These PCR primers were used to amplify the CAST gene. An aliquot of 5µl 100ng genomic DNA was amplified in total volume of 20µl PCR mix. The PCR mix consisted of: 7µl of 2X Red PCR Master Mix, 2µl of Forward Primer (10 pmol/µl) and Reverse Primer (10 pmol/µl) and 4µl of ddH_2O. The tubes were placed in a Master cycler gradient (Bio Rad S1000, USA) and subjected to PCR. The PCR protocol was as follows: pre denaturation at 94°C for 5 min, followed by 34 cycles of denaturing at 94°C for 35 s, annealing at 55.0°C for 45 s, extension at 72°C for 1 min, and final extension at 72°C for 5 min.

Gel electrophoresis

Intensified PCR products were verified on 1.5 % agarose gel along with 1kb DNA ladder at a invariable voltage of 70 V for 45min to1 hour in 1X TBE buffer. Ethidium bromide was used to stain the gel. The gel was envisioned under the UV transilluminator (Gel documentation system–Bio rad Molecular imager Gel Doc XR+, USA).

Sequencing and Data analysis

PCR products were purified and sequenced commercially from The Advance Bio Science International. Data were analyzed on ensemble genome browser by Blast the query sequence of cast gene of selected breeds with the genomic sequence of cast gene.

Results and discussion

In this study, we have found mutations with the help of PCR-Gel Electrophoresis and DNA sequencing technique.

PCR-Gel Electrophoresis

A 174-bp portion of the amplified product was PCR amplified in all breeds (Figure 1). After PCR amplification and sequencing of a 174-bp fragment (figure 2), we discovered mutation based on genetic code.

Table 1. Details about the oligonucleotide PCR primers.

Primer	Forward prime 5′ - 3′	Reverse prime 5′ - 3′	bp	Tm
Cast gene	TCCAGAACTCAGGCTGATGA	CCACCTGCCCAGAAATGATA	174	55

Table 2. Mutations identified based on Genetic code**.**

Sample name	Number of change nitrogenous base	Original codon	Changed codon	Original amino acid	Changed amino acid	Type of Mutation
CAST. S2	27	AAA	ACA	Lysine (E)	Theronine (E)	Missense Mutation
	41	ATC	AGC	Isolucine (E)	Serine(N)	Missense Mutation
CAST. RS	60	TGT	CGT	Cystine (N)	Arginine(E)	Missense Mutation
	80	CAA	CAG	Glutamine(N)	Glutamine(N)	Silent Mutation
CAST. TH2	35	GTA	GTG	valine(E)	valine(E)	Silent Mutation
	57	GAA	AAA	Glutamine(N)	Lysine (E)	Missense Mutation
CAST. TH5	125	TAT	TGT	Theronine(E)	Cystine (N)	Missense Mutation

Mutation based on genetic code

In this study, we have found total 7 mutations with the help of PCR-Gel Electrophoresis and DNA sequencing technique presented in table 2 and Percentage of mutation among three cattle breeds presented in table 3. Based on Genetic code, we have

identified (02) mutations in Exon-5 of Red Sindhi cattle breed including a silent mutation at 80 bp in which CAA(Glutamine) codon was changed to CAG(Gluatmine) but does not causes any change of Amino acid hence will not affect on meat qualities of the Red Sindhi cattle.

Table 3. Percentage of mutation determined among three cattle breeds.

Name of Gene	Name of Breed	Found SNP	Percentage method	Total percentage
CAST	Sahiwal	2	2/174×100	1.149%
	Red sindhi	2	2/174×100	1.149%
	Tharparkar	3	3/174×100	1.724%

Another mutation was also found in Exon 5 of the Red Sindhi cattle breed, which causes change of codon from TGT to CGT, in which former was coding for the Cystine and later is coding for Arginine Amino acid change from non essential to essential. In Sahiwali cattle, 02 mutations were also identified in exon 3, in which AAA codon was changed to ACA at 27 bp, which results in substitution of Lysine to Threonine amino acid. Second mutation caused the change of codon from ATC to AGC which results in change of amino acid from Isoleucine to serine amino acid. We have also identified 03 mutations in Exon5 of Tharparkar cattle breed First one was identified at 35 bp of Exon 5, in which GTA codon was changed to GTG, which does not cause any change of amino acid so know as silent mutation.(Valine to Valine) Second mutation was identified at 57 bp in which GAA codon was changed into AAA, which results in change of Glutamine amino acid to Lysine Amino acid. Third mutation resulted in change of TAT codon to TGT codon that caused change of Thyronine amino acid to Cysteine amino acid.

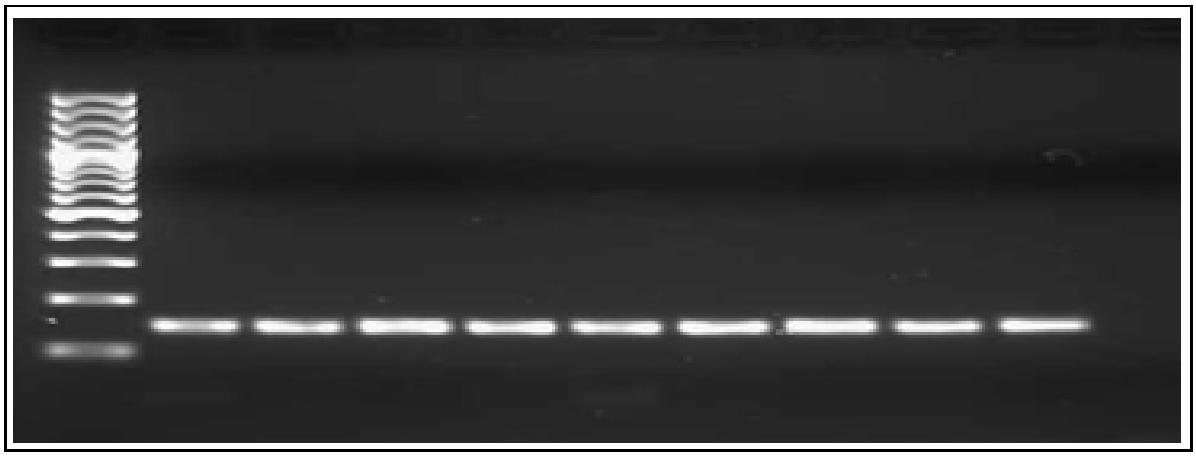

Fig. 1. PCR product of cast gene.

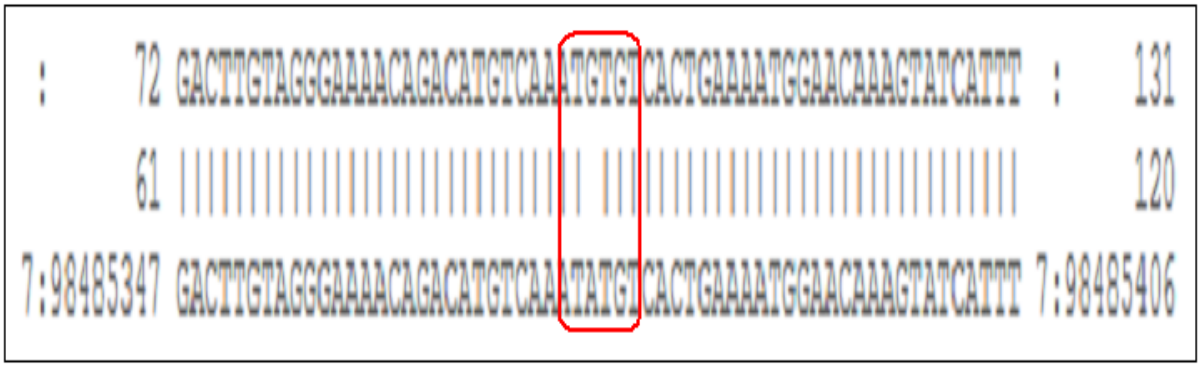

Fig. 2. Sequence alignment of the bovine CAST gene.

Conclusion

In conclusion, Essential mutation can be effect the meat quality and quantity in position manner and can be used in Marker Assisted selection. Some mutations were not associated with tenderness, but they were associated with cooking loss, color scores, fatty acid content, and amino acid content. Validation depends on the specific nature of the population screened and the genetic background may influence the effects of polymorphisms.

For the association's confirmed in the current study, the additional validation suggests that these markers can be used in selection programs for improving the meat quality of livestock of these cattle breed.

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