IJAS Iranian Journal of Applied Animal Science

Allelic Polymorphism of Calpastatin Gene (CAST) in Khalkhali Goats: A Possible Marker for Meat Tenderness

Research Article

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Received on: 24 Jun 2014
Revised on: 26 Nov 2014
Accepted on: 30 Jan 2015
Online Published on: Dec 2015

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Online version is available on: www.ijas.ir

ABSTRACT

Calpastatin (CAST) is a specific inhibiter of Calpains, playing a role in meat tenderization and myogenesis. In the present study the polymorphism of the CAST gene of Khalkhali goat in Azerbaijan province in Iran was investigated by polymerase chain reaction and restriction fragment length polymorphism technique (PCR-RFLP). Genomic DNA was extracted from whole blood samples collected from 200 Khalkhali goats. A 1552 bp in the region between exons 6 and 7 of the CAST gene was amplified by standard PCR, using the locus specific primers. Two alleles (A and B) and three genotypes (AA, BB and AB) were observed. The frequencies of the observed genotypes were 49.7, 0.09 and 41.4 for AA, BB and AB, respectively. Allele frequencies were 0.705, 0.295 for A and B, respectively. The average heterozygosity for CAST gene was 0.416 and the expected heterozygosity was 0.417. For homozygosity these numbers were 0.91 and 0583, respectively and the chi-square test showed significant ($P<0.01$) deviation from Hardy-Weinberg equilibrium for this locus in this population.

KEY WORDS CAST gene, Khalkhali goat, PCR-RFLP.

INTRODUCTION

The improvement in meat quality is one the main goal of livestock production and meat tenderness is one of the most important factors for quality assessment of the meat. The Calpain proteolytic system has been identified as a factor for the postmortem meat tenderization process through the proteolysis of myofibrillar and associated proteins (Koohmaraie, 1992; Taylor *et al*. 1995). It is also involved in the regulation of myoblast migration and fusion (Barnoy *et al*. 1996; Dedieu *et al*. 2004), protein turn-over and muscle growth (Huang and Forsberg, 1998). Variation in meat tenderness is due the genetic variation, biological and physiological differences during slaughter, and chemical differences during the post-mortem aging (Koohmaraie, 1996). Calpastatin (CAST) gene is located on the fifth chromosome of sheep and encodes a specific Calpain inhibitor which plays important roles in formation of muscle, degradation and meat tenderness after slaughter (Huang and Forsberg 1998; Palmer *et al*. 1999). In the bovine (Casas *et al*. 2006; Schenkel *et al*. 2006) and pigs (Ciobanu *et al*. 2004) CAST gene polymorphisms have been associated with meat tenderness, making the CAST gene an excellent candidate for controlling meat tenderness. Assessment of the genetic polymorphism in CAST gene and its relation to the meat quality could be used as a tool to predict meat ten-

derness in animals allowing breeders to enhance the trait (Seiler, 1994). Marker assisted selection is an efficient DNA based methods that improves accuracy and progress of selection in animal stock (Koohmaraie, 1996). In addition, genotyping animals by employing this molecular marker will help to classify carcasses based on eating quality before slaughter (Lonergan *et al*. 1995). It was demonstrated that the favorable effect of the variants of CAST gene on pig carcass quality traits depends on the cut. It was also reported that post-mortem changes in different periods depends on the *CAST*/*RsaI* genotype. It seems that the BB genotype is related to the rate of glycolysis immediately after slaughter while the AA genotype is related to the rate of glycogenolysis in the process of conversion muscles into meat (Krzecio *et al*. 2008).

Palmer *et al*. (1998) described two allelic systems of the polymorphic variants (M and N) in a region of the bovine CAST by PCR-RFLP method. Using a molecular genetics approach to study meat quality in sheep, Palmer *et al*. (1999) have chosen the ovine CAST gene as a candidate gene for meat quality. A three allelic system of the polymorphic variants (a, b and c) by PCR and a single strand conformation polymorphism (PCR-SSCP) in a region of the ovine and cattle CAST was observed (Chung *et al*. 1999; Palmer *et al*. 2000).

In the present study we aimed to evaluate the genotype and genetic frequencies of CAST gene in Khalkhali goats of Azerbaijan province, north-western of Iran, by PCR-RFLP technique. To date there has been no report on the allelic polymorphism of CAST gene, in any of the caprine breeds from north-western Iran. Results of our study can be further associated with the quality of meat in these breeds and can be used as a plausible marker for prediction of meat tenderness in related breeds.

MATERIALS AND METHODS

Caprine, blood sample collection and genomic DNA extraction

Handred males with average weight of 52.5 ± 2.5 kg and 100 females with average weight of 42.5 ± 2.5 kg of Khalkali goats were enrolled and all of them were horned, when animals was in the ranged of 9 month to 2 years. The most mass distribution of this breed is around of Khalkhal city and some parts of East Azarbaijan, Iran.

Approximately 3 mL of blood samples were obtained from 200 unrelated Khalkhali goats from different parts of Azerbaijan province and stored in EDTA-coated tubes. Genomic DNA was extracted from 0.3 mL blood using the genomic DNA purification kit (Fermentas, EU) according to manufacturer's instructions. Quality and quantity of extracted DNA was measured by agarose gel (2 %).

Amplification of the region between exon 6 and 7 of CAST gene

The DNA amplification of the CAST gene was achieved by PCR. PCR-RFLP genotyping was used to detect the polymorphism in the region between exons 6 and 7 of the CAST gene. This method is an easy and reliable one which has been commonly used for detecting polymorphism of CAST gene in different populations. So we used this methodology to give a comparative description of our results with those of other population. Two primers (Forward primer: 5'-AGCAGCCACCATCAGAGAAA-3' and reversed primer: 5'-TCAGCTGGTTCGGCAGAT-3' targeting a fragment of 1552 bp was employed as described by Chung *et al*. (2001).

The PCRs were carried out in 50 µL volumes using PCR mastermix kit (Cinnagen, Iran) containing 2.5 units of Taq DNA Polymerase in reaction buffer, 4 m*M* $MgCl_2$, 50 µM each of dATP, dCTP, dGTP and dTTP, 0.5 µM of each primer and about 100 ng of extracted DNA as template. The PCR protocol included an initial denaturation at 94 ˚C for 5 min, 35 cycles of denaturation at 94 ˚C for 1 min, annealing at 57 ˚C for 1 min and polymerization at 72 ˚C for 2 min, and a final extension at 72 ˚C for 5 min. The amplification was carried out using a Mastercycler (Eppendorf, Germany).

Restriction fragment length polymorphism (RFLP)

Eight microlitres of the PCR product was digested with 10 units of the restriction endonucleases *XmnI* at 37 ˚C for 14 h. The electrophoresis was performed in 0.5 X TBE buffer (Tris 100 m*M*, Boric Acid 9 m*M*, EDTA 1 m*M*) at room temperature (18 ˚C) and constant 120 V for 90 min in 2% agarose gels, stained with ethidium bromide and viewed under UV light.

Statistical analysis

The allelic and genotypic frequencies, expected means, observed and expected Nei's heterozygosities (19):

$$HE = 1 - \Sigma P_i^2$$

Where:

P_i: frequency of allele.

i: Hardy-Weinberg equilibrium were calculated using PopGene32 program, version 1.31, Canada (Yeh *et al*. 1997).

RESULTS AND DISCUSSION

PCR-RFLP analysis of CAST gene

The amplification of a 1552 bp fragment of the region between exon 6 and 7 of the CAST gene was successfully produced in our first attempt. All extracted DNAs from Khalkhali goat blood samples yielded a specific single band

PCR product without any nonspecific band. Therefore, the PCR products were directly used for RFLP analysis (Figure 1).

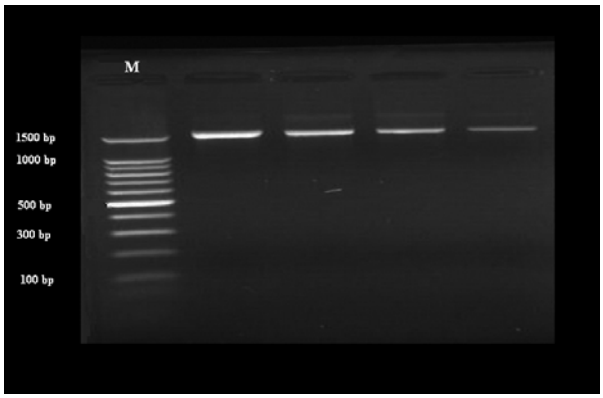

Figure 1 PCR products
Lane M shows the 100 bp molecular weight marker

The allelic variation in the CAST gene was examined by PCR-RFLP. The PCR-RFLP technique revealed two alleles; allele A was the PCR product with the restriction site for *XmnI* which upon digestion produced two fragments of approximately 960 and 592 bp and allele B was the undigested 1552 bp PCR product (Figure 2).

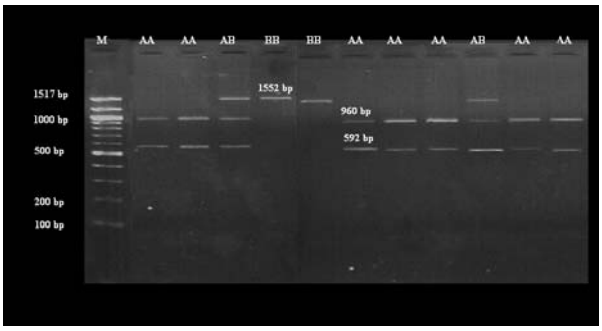

Figure 2 RFLP polymorphism of Caprine CAST gene
M shows the 100 bp molecular weight marker
Three different PCR-RFLP patterns (genotype) were identified (AA, AB and BB)

In this study, a total of three genotypes were observed in the examined population. The frequencies of the observed genotypes were 49.7, 0.09 and 41.4 for AA, BB and AB, respectively. Allele frequencies were 0.705 and 0.295 for A, and B, respectively (Table 1). The heterozygosity average for CAST gene was 0.416. The chi-square test showed significant ($P<0.01$) deviation from the Hardy-Weinberg equilibrium for this locus in the investigated population.

In the present study two alleles (A and B) and three genotypes (AA, BB and AB) were observed for CAST gene in Khalkhali goat in Azerbaijan province, Iran. The most frequent allele and genotype in the Khalkhali goat were 70.5 and 29.5% for allele A and allele B, respectively (Table 2). The results obtained from this study revealed the polymorphism pattern of the CAST gene in Khalkhali goats.

Table 1 Observed alleles and genotypic frequencies for *CAST* gene in Khalkhali goats

A	B	AA	BB	AB
0.705	0.295	49.7	0.09	41.4

Table 2 Estimated statistically parameters for *CAST* gene in Khalkhali gots

Exp-Het	Exp-Hom	$Het_{(Nei)}$	Ave-Het	Obs-Hom	Obs-Het
0.417	0.583	0.416	0.416	0.91	0.09

Several methods including PCR-RFLP, PCR-SSCP and sequencing have been used to investigate the polymorphism in the CAST gene in domestic animals. Variation in non-coding and coding regions of the ovine CAST gene has been reported by several researchers (Palmer *et al*. 1998; Palmer *et al*. 2000; Roberts *et al*. 1996; Zhou *et al*. 2007). There is very limited study on CAST gene in goats although there are many reports available for cattle. Zhou and Hickford (Zhou and Hickford, 2008), based on PCR-SSCP analysis of the Caprine CAST gene, observed high homology with published ovine and bovine CAST sequences. Study of polymorphism on the same region of the CAST gene in Kurdi sheep by PCR-SSCP revealed three genotypes including AA, AB and AC (Nassiry *et al*. 2006). The polymorphism in the exon 1 of the CAST in sheep was also reported by the other researchers using PCR-RFLP technique (Gabor *et al*. 2009; Mohammadi *et al*. 2008; Palmer, 1998). In goats and bovine, the exon 6 of CAST gene was investigated for polymorphisms and a number of allelic variants were identified in these species (Zhou *et al*. 2007; Zhou and Hickford, 2008). Some studies reported higher frequencies of CAST gene's allele A compared to the allele B in Nellore (0.66), Rubia Gallega (0.72), Canchim (0.62), Brangus (0.78) and Pardo Suico (0.80) cattle. There are several studies on the association of CAST gene polymorphism with meat quality by PCR-RFLP analysis in animals. Schenkel *et al*. (2006) reported a significant association between allele C of bovine CAST gene and the meat tenderness. In addition, Kuryl *et al*. (2003) reported that CAST gene may be considered as a candidate gene for pig carcass quality. Association between allele D and F of porcine CAST gene and meat quality traits was also reported by Kapelański *et al*. (2004).

Palmer *et al*. (1999) found allelic frequencies of 0.69 and 0.70 for allele A in Dorset Down and Coop worth, respectively, which it was in close agreement with the frequency of the allele A in the present study. In contrast, they found that frequencies of alleles A and B in Corriedale and Ruakura were 0.27 and 0.41, respectively. Different frequencies for the alleles of the CAST gene have been reported in Ira-

nian Baluchi sheep with 0.70 for allele A, 0.08 for allele B and 0.22 for allele C.

Genotypes BC and CC, which presented the 0.03 and 0.04 frequencies, respectively in Baluchi sheep, were not observed in Khalkhali goat (Tahmoorespur *et al*. 2007). Two allelic systems of polymorphic variants (M and N) in the region of ovine CAST locus have been described by PCR-RFLP method (Palmer, 1998; Eftekhari Shahroodi *et al*. 2005; Elyasi-Zaringhabaee *et al*. 2005). According to Palmer *et al*. (1998), allelic frequencies were 77% and 12% for the M and N in Corriedale sheep, respectively.

CONCLUSION

This study was the first attempt for identification of CAST gene variation in Khalkhali goats. Two alleles (A and B) and three genotypes (AA, BB and AB) were observed in the region between exons 6 and 7 of the CAST gene in Khalkhali goats that bred in Azerbaijan province of Iran. The most frequent allele and genotype in the Khalkhali goat breed were 70.5% and 29.5% for allele A and allele B, respectively. Our results can be expanded by further comparing these observed polymorphisms of CAST gene with performance traits like meat tenderness, in Khalkhali goats. The functional role of the polymorphic sites in this Caprine needs to be analyzed and confirmed by means of gene expression assays.

ACKNOWLEDGEMENT

Authors sincerely thank dean of research of Uroomia University for funding this project. They also thank Mr. Pouya Mohammadi for their technical assistance.

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