Genetic Variability of CAST Gene in Native Sheep Breeds of Turkey [1]

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Abstract

The aim of this study is to determine the genetic variability of CAST gene in native sheep breeds of Turkey by PCR-RFLP method. Six different native sheep breeds; Kivircik, Imroz, Karayaka, Hemsin, Red Karaman and Karakul were used in this study. This study was the first report about CAST gene variation in Karayaka, Red Karaman and Hemsin sheep breeds. After DNA isolation and PCR amplification, RFLP was performed with MspI enzyme. Two alleles M (336bp and 286bp) and N (622bp) were identified on 2% agarose gel electrophoresis. Allel and genotype frequencies, observed (Ho) and expected heterozygosity (He) and deviation from Hardy Weinberg Equilibrium were estimated by statistical analyses. The frequency of M allele was highest in Imroz (96%) and N allele was identified most frequently in Kivircik (30%) breed. Highest frequencies of MN genotype were identified in Kivircik (30%) and Imroz (92.6%) breeds respectively. Kivircik, Imroz, Karayaka and Karakul breeds were null from NN genotype. Kivircik sheep showed the highest heterozygosity (60%) and Imroz had the lowest (7.4%). The highest heterozygosity value was identified in Kivircik (60%), the lowest in Imroz (7.4%). All breeds except Kivircik and Hemsin were found in Hardy-Weinberg equilibrium. Absence of NN genotype in some breeds and high frequency of MN genotype in Kivircik breed might be resulted from the selection process of native sheep breeds in their breeding regions.

Keywords: Calpastatin, Native sheep breeds, Genetic variation

Türkiye Yerli Koyun Irklarında CAST Genine Ait Genetik Çeşitliliğin Belirlenmesi

Özet


Anahtar sözcükler: Kalpastatin, Yerli koyun, Genetik çeşitlilik

INTRODUCTION

Sheep is one of the most important red meat sources in Turkey [1]. Calpastatin (CAST) gene captures special attention for its major role in both meat tenderness and growth in animals. Therefore CAST is one of the most screened genes in livestock. Various studies have been performed to identify the CAST gene variation in goats [2] and its association with meat quality traits in pigs [3] and cattle [4-7]. CAST gene was first identified in sheep by...
Palmer et al. [8] and it was located on the 5th chromosome in sheep genome [9]. Calpastatin (CAST) enzyme is the specific inhibitor of calpain proteases which regulates the rate and extent of post mortem tenderization [10]. Calpain enzyme plays a key role in meat tenderness by degrading myofibrillar proteins after slaughter during the process of rigor mortis [11]. Calpain CAST system (CCS) is important in muscle growth. Reduction of calpain and increase in CAST activities may result in increase in growth rate of skeletal muscle. CAST gene was described as an important regulator on birth weight; its influence was shown on growth rate until weaning in Romney lambs [12]. Chung and Davis [13] reported that CAST gene has positive effect on both average daily gain and post weaning weight in Targhee sheep. However Dehnavi et al. [14] did not find any relation between CAST gene and yearling weight in Zel sheep. Variation of CAST locus in various sheep breeds were identified by using PCR-RFLP [10,14-26], PCR SSCP [9,27-29] and DNA sequencing [27,30-32] methods. A point mutation in intron 12 region of CAST gene causes the substitution of Guanin (G) nucleotide to Adenine (A) and diverges the CCGG nucleotides to CCAG sequence. Since CCGG is recognition site for MspI enzyme, G-A substitution makes the site unrecognizable by the enzyme; therefore the mutation can be identified with PCR-RFLP method [31]. Two alleles (M and N) and three genotypes (MM, MN and NN) were described in CAST locus after PCR-RFLP analysis with MspI enzyme [30].

The aim of this study was to identify CAST genotype variation in thin-tailed sheep breeds; Kivircik, Imroz and Karayaka, semi-fat tailed breed; Hemsin and fat-tailed sheep breeds; Red Karaman and Karakul by using PCR-RFLP method.

**MATERIAL and METHODS**

This study was approved by Ethic Committee of the Istanbul University Veterinary Faculty (Approval number: 2011/163).

Thin-tailed sheep breeds; Kivircik (n=25), Imroz (n=27) and Karayaka (n=22), semi-fat tailed breed; Hemsin (n=19) and fat-tailed sheep breeds; Red Karaman (n=14) and Karakul (n=15), in total 122 sheep were used as animal samples. Blood samples were taken from Vena jugularis into sterile vacuumed EDTA tubes in five different sheep breeds. Genomic DNA was isolated from blood by using ExiPrep™ 16Plus automated nucleic acid extraction system (Bioneer Company, Chonbuk, Chonju, South Korea). DNA isolation in Karayaka sheep breed was performed from raw meat samples. DNA from meat samples were obtained by PureLink DNA isolation kit (Invitrogen, Carlsbad, CA, USA).

The region of the ovine CAST gene was amplified by using PCR with the forward primer 5’TGGGGCCCAATGACCGCATTGCATG3’ and the reverse primer 5’GTTGGAGCATCGATTGCAC3’, which captured a 622bp sized fragment from intron 12 and exon 13 (AF016006.1) [8,31].

PCR was carried out in a final volume of 50 µl containing; 100 ng genomic DNA, 20 pmol each primer, 200 mM dNTPs each, 1.5 mM MgCl₂, 10X PCR Buffer and 0.25U Taq polymerase (MBI Fermentas). PCR was performed with the following conditions; denaturing at 95°C in 3 min, 35 cycles of 95°C in 30 sec, 63°C in 50 sec, 72°C in 1 min and final extension at 72°C in 10 min (Bio-Rad T100, Bio-Rad Laboratories Inc., CA, USA). PCR products were digested with 1 µl (10U) MspI enzyme (MBI Fermentas). Samples were incubated at 37°C by overnight for MspI digestion. After performing RFLP, band patterns were visualized by 2% agarose gel.

Samples showed polymorphic band pattern for MspI enzyme were sequenced both forward and reverse directions by REFGEN gene research and biotechnology firm (www.refgen.com) with ABI 3100 avant automated DNA sequencer in order to confirm the haplotypes.

Allele and genotype frequency observed and expected heterozygosity and chi square test to analyze the deviation from Hardy-Weinberg equilibrium (HWE) were estimated by using PopGene32 software program version 1.31 [31].

The sequence results were compared with the reference sequence of ovine CAST gene (GenBank: AF016006.1) by using BioEdit sequence alignment editor (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) software program.

**RESULTS**

Two alleles of CAST locus (M and N) were identified after digestion with MspI enzyme. Band patterns of M allele (336bp and 286bp) and N allele (622bp), MM, MN and NN genotypes were viewed on 2% agarose gel stained with EtBr (Fig. 1).

Allele frequencies, genotype frequencies, observed and expected heterozygosity, chi square and p values of CAST locus were given in Table 1.

Three different haplotypes; a (CCGG), b (CCGGA) and c (CCAGA) were identified for CAST locus (Fig. 2-A). Haplotypes that constitute MM (aa, ab), MN (ac) and NN (cc) genotypes were shown in Fig. 2-B. None of the MN genotype showed haplotype b. Only Hemsin and Red Karaman breed were carry haplotype b within their MM genotype.

**DISCUSSION**

The frequency of M allele was the highest in Imroz (96%) sheep. N allele was identified most frequently in Kivircik (30%) breed. The highest frequencies of MN genotype was observed in Kivircik (60%), MM in Imroz (92.6%) and NN in Red Karaman (7.1%) breeds respectively.
Previous studies performed by other researchers for identifying genetic variation of CAST gene in different sheep breeds with RFLP method were summarized in Table 2.

According to the results of this study M and N allele frequencies and MM and MN genotype frequencies of Imroz sheep was found similar with Ile de France breed [22]. M and N allele frequencies of Red Karaman sheep were...
found similar with Cine Capari [26] and Polish Merino breed [22]. MM genotype frequency was also found similar with Polish Merino breed [22]. Observed heterozygosity and expected heterozygosity values of Red Karaman breed were found similar to both Iranian Karakul [24] and Cine Capari breed [26] respectively. M and N allele frequencies and MN genotype frequency of Karakul sheep were found similar with Cine Capari sheep breed [24]. Khan et al. [18] found that heterozygous (MN) genotype showed significantly higher weight gain from birth to eight months in Balkhi sheep and from birth to four months of age in Kajli sheep breeds respectively. Results of this study showed that Kivircik, Imroz, Karayaka and Karakul breeds were found null from [17].

Khan et al. [18] also reported that animals with NN genotype showed lower average daily gain (ADG), back fat thickness (BT) and skin with back fat thickness (S+BT) values.

It can be concluded from the current study that, selection process of native sheep breeds in their breeding
regions may occurred negatively for NN genotype in Kivircik, Imroz, Karayaka and Karakul sheep breeds however it may occurred positively for MN genotype in Kivircik breed. Kivircik is the most popular sheep breed for red meat source in Turkey.

Imroz, Red Karaman, Karayaka and Karakul populations were found in HWE. However Kivircik and Hemsin were not in HWE being similar to Kivircik [16], Dalagh [10] and Zel sheep [14] populations. The highest and lowest heterozygosity values were identified in Kivircik (60%) and Imroz (7.4%) breeds respectively. Since Imroz breed is originated from Imroz Island, low heterozygosity value is an expected outcome for this breed.

CAST gene haplotypes obtained with sequencing, were found very similar with the findings of Gregula-Kania [31]; only CAST-B haplotype (CCGGA) was divergent than reported data. CAST B haplotype located in border of recognition site of MspI enzyme, was identified only in MM genotype of Hemsin and Red Karaman breeds. However structure of M and N alleles were formed respectively by haplotype a and c which were localized in MspI recognition site.

Understanding the effect and selection variation of CAST locus may help to improve marker assisted selection (MAS) studies in sheep breeding, particularly in meat tenderness and growth. This was the first report about CAST gene variation in Karayaka, Red Karaman and Hemsin sheep breeds of Turkey. Further studies on CAST locus should be performed in different native sheep breeds to enlighten the genotype structure and candidacy profile for MAS studies of sheep genetic resources of Turkey.

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