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Polymorphism of the ovine calpastatin gene in some Turkish sheep breeds

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Abstract: The calpastatin (*CAST*) gene has a major effect on muscle growth and meat tenderness after slaughter; it is located on the fifth chromosome in sheep. Blood samples were collected from 720 animals in total from Kıvırcık (KIV), Sakız (SZ), Karacabey Merino (KM), and Gökçeada (GA) sheep populations raised in West Anatolia. The PCR products were digested by the restriction endonuclease *MspI*. Allele frequencies for M and N alleles of the gene were found to be 0.85 and 0.15 in KIV, 0.80 and 0.20 in KM, 0.99 and 0.01 in GA, and 0.34 and 0.66 in SZ sheep, respectively. It was determined that NN genotype frequency was quite lower in KIV (0.04) and KM (0.07) populations than in the SZ breed (0.40). On the other hand, the NN genotype was not observed in the GA population. Populations other than KM were found to be in Hardy–Weinberg equilibrium. In general, allele and genotype frequencies of *CAST* were similar to those in other studies on different sheep populations. This research is a beginning step for finding candidate genes' effects on meat quantity and quality.

Key words: Calpastatin, sheep, PCR-RFLP, *MspI*

1. Introduction

In recent years, many quantitative trait loci (QTLs) having effects on economically important traits have been determined in farm animals as a result of advances in molecular genetics. One of the QTLs identified is the calpastatin (*CAST*) gene, located on the fifth chromosome of sheep (1). *CAST* is the endogenous and specific inhibitor of calpains; it inhibits the calpain activity in postmortem tissue and thus regulates the rate and extent of postmortem meat tenderization (2). The majority of studies showed that *CAST* may be a significant gene with respect to the carcass and the characteristics of meat quality (3,4). M and N alleles belonging to the *CAST* gene, as reported initially by Palmer et al. (1), were identified using 2 different restriction enzymes. The presence of these alleles was determined in other studies also conducted on other sheep populations (5–14).

Lamb meat production is the main objective in sheep breeding in the western part of Anatolia. Therefore, the quality of produced meat is quite significant. Accordingly, identification of the *CAST* gene, known to have significant effects on meat quality, in these breeds will contribute to the literature. Additionally, *CAST* polymorphism is an important area of research, given that it has not been widely investigated in Turkish sheep breeds.

The object of the study was to show polymorphism of the *CAST* gene, which might be a candidate gene for genomic selection studies in the future, in Gökçeada (GA), Kıvırcık (KIV), Karacabey Merino (KM), and Sakız (SZ) sheep populations raised in West Anatolia by the method of PCR-RFLP. Studies including molecular genetics and performance data (growth traits, meat quality, etc.) will strengthen the breeding programs for these breeds. In addition, genotyping animals by employing this molecular marker will help to classify carcasses based on meat quality before slaughter.

2. Materials and methods

2.1. Blood samples

Blood samples were randomly collected from 720 lambs born in 2013 belonging to 4 different sheep breeds (GA, KIV, KM, and SZ) reared in West Anatolia. Approximately 4.5 mL blood samples were gathered from the vena jugularis in K3-EDTA tubes and transferred to a –20 °C freezer. The location and sample sizes of these 4 breeds are given in Table 1.

2.2. DNA isolation

The DNA was isolated with a DNA isolation kit (Applied Biological Materials Column-Pure Blood Genomic DNA Kit, Canada) from blood samples as per the manufacturer's instructions in the Adnan Menderes

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Table 1. Locations and sample sizes of sampled populations of GA, KIV, KM, and SZ sheep breeds.

Breeds	N	Breeding location	Flock type	Farms
GA	49	SRS	GRCF	1
KIV	336	Uşak, Turkey	Breeders Farm	12
KM	248	SRS	Nucleus Flock	1
SZ	87	SRS - ADU-GKYP	GRCF	2

SRS: Sheep Research Station, Bandırma, Turkey; ADU-GKYP: Adnan Menderes University Group Sheep Breeding Program, Aydın, Turkey; GRCF: genetic resource conservation flock.

University Faculty of Agriculture Department of Animal Science, Genetics Laboratory, Aydın, Turkey. Quantity and quality of the DNA were checked using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA).

2.3. Amplifications of DNA fragments

The DNA amplification of the *CAST* gene was achieved by PCR-RFLP. Two primer pairs [5'-CCT TGT CAT CAG ACT TCA CC-3' (forward) and 5'-ACT GAG CTT TTA AAG CCT CT-3' (reverse)] targeting a fragment of 565 bp were employed as described by Khederzadeh (8) for identification of the M and N alleles of *CAST*.

The PCR amplification reaction solution was performed in total volume of 25 µL containing ddH_2O, dNTP (0.2 mM), $MgCl_2$ (2.0 mM), primers (0.25 µM), PCR buffer (1X), Taq DNA polymerase (1 U/µL), and template DNA (~100 ng). The PCR cycling condition was a preliminary denaturizing at 92 °C for 2 min, followed by 1 cycle of denaturing at 92 °C for 1 min, annealing at 65 °C for 1 min, and extension at 72 °C for 2 min followed by 35 cycles and 10 min at 72 °C as a final extension. The PCR reactions were performed on an ABI Veriti thermocycler.

2.4. PCR-RFLP genotyping

The amplified fragment of *CAST* was digested by the restriction endonuclease *MspI* (Fermentas). Digestion was conducted at 37 °C for 6 h and in a 30-µL reaction solution including 0.50 µL of ddH_2O, 3.00 µL of 10X Buffer Tango, 1.50 µL of *MspI*, and 25 µL of PCR product. Digested products were separated by electrophoresis on 2% (v/w) agarose gel stained with Safe View (NBS Biologicals, UK) (Figure). Electrophoresis was performed in a 0.5X TBE buffer at room temperature and constant 65 V for 120 min.

2.5. Statistical analysis

Allelic and genotypic frequencies, observed and expected heterozygosity, and Hardy–Weinberg equilibrium were calculated using GenAlEx (15) and PopGene 32 (16) programs.

3. Results

Allele frequencies, genotype frequencies, and observed (Ho) and expected (He) heterozygosity values obtained from the study and the results of chi-square tests performed for Hardy–Weinberg equilibrium are presented in Table 2.

The highest value for the M allele was found in the GA population (98.98%) while the lowest was seen in the SZ population (34.48%). Considering all populations with respect to the N allele, as a significant result the highest frequency was found in the SZ population (65.52%) and the lowest was observed in the GA population (1.02%). The M allele was the most common in the studied sheep populations, except for the SZ breed. According to genotype frequency assessments, the NN genotype could not be identified in the GA sheep breed, while the MM genotype was determined as the most

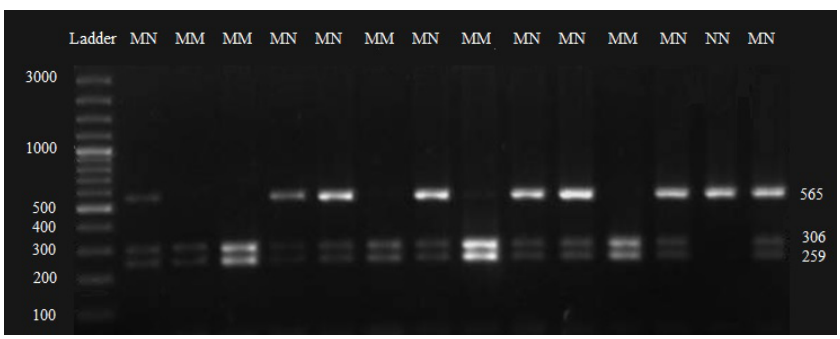

Figure. Gel image for the ovine *CAST* genotypes.

Table 2. Allele and genotype frequencies, observed (Ho) and expected (He) heterozygosity, and Hardy–Weinberg equation (χ^2) for *CAST* loci.

Breeds	N	Allele freq. (%)		Genotype freq. (%)			Heterozygosity		
		M	N	MM	MN	NN	Ho	He	χ^2
KIV	336	84.67	15.33	72.92	23.51	3.57	0.235	0.260	2.980^{NS}
KM	248	80.04	19.96	66.94	26.21	6.85	0.262	0.320	8.009**
SZ	87	34.48	65.52	9.20	50.57	40.23	0.506	0.452	1.238^{NS}
GA	49	98.98	1.02	97.96	2.04	0.00	0.020	0.020	0.005^{NS}

**: $P < 0.01$, NS: nonsignificant.

common genotype among all populations in this study. The highest genotype frequency was obtained in SZ population (40.23%) with respect to the NN genotype (Table 2). Hardy–Weinberg equilibrium was examined for all populations and only the KM population was in disequilibrium ($P < 0.01$).

4. Discussion

M and N alleles belonging to *CAST* were identified in all studied populations. Frequency values obtained from M and N alleles in the KIV and KM populations were similar to those in the relevant literature (5–14,17). The results showed that the most common allele and genotype in the KIV, KM, and GA populations were M and MM, respectively.

The KM and KIV populations are among the most common populations bred in Turkey due to their meat production and quality. Chung and Davis (18) reported that the *CAST* gene has an effect on birth weight and average daily live weight gain. Similarly, Khan et al. (7) reported that animals having the NN genotype have lower live weight averages than others. Assessing the obtained data for these populations in light of the literature raises suspicion that there is selection developing against the NN genotype, having unfavorable effect on live weight. In fact, a selection program targeting live weight was implemented many years ago under the scope of the National Merino Project in the KM population. Especially in the KM population, the fact that allele distributions of the *CAST* gene are not in Hardy–Weinberg equilibrium supports the impact of an intensive selection program.

N allele and NN genotype frequencies in the SZ breed in the present study were quite higher than in the relevant literature. The SZ sheep were from a conservation flock, which is relatively more isolated than the other studied populations. Thus, it may be thought that inbreeding has increased in this population over the course of time and raised certain genotype frequencies. Similar allele and genotype frequencies in SZ populations were reported in a study by Yılmaz et al. (19) conducted on 10 domestic Turkish sheep populations.

Although the N allele was observed at low frequency in the GA population, the NN genotype could not be observed. This agreed with the results of Gabor et al. (5) in Ost Friz sheep. Furthermore, the frequency of the MN genotype is also quite low in this population. This indicates that homozygosity has occurred in the population with respect to M allele.

The obtained results are similar to those found in studies conducted on different sheep populations with respect to allele and genotype frequencies of the *CAST* gene. This shows that quite important information on the relation between *CAST* and meat quality can be provided by performing studies focused on meat quality and molecular genetics.

CAST can be used as a significant marker in selection programs aiming to improve meat quality and production with the information obtained from such studies. The findings from these studies can be used in selection programs, ongoing for many years, targeting live weight and meat quality in KIV and KM sheep.

Genetic selection of superior animals for meat tenderness could be made more efficient by genotyping animals for the *CAST* locus. Conducting studies of performance data (growth characteristics and meat quality), especially in the manner of improvement of meat yield and quality, and genetic research will significantly contribute to future genomic selection works.

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