

## Genetic Polymorphism of Five Genes Associated with Meat Production Traits in Five Cattle Breeds in Turkey

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Article Code: KVFD-2014-12722 Received: 03.12.2014 Accepted: 26.02.2015 Published Online: 17.03.2015

### Abstract

There are many potential genes that are known to be related to meat production and that can be used for selection to improve meat quality and production include Calpain (CAPN1), Myostatin (MSTN), Calpastatin (CAST), Osteopontin (SPP1) and Thyroglobulin (TG). This study evaluated Zavot (n=60), Anatolian Black (n=59; AB), South Anatolian Red (n=53; SAR), Turkish Gray (n=60; TG) and East Anatolian Red (n=49; EAR) cattle breeds, with the primary goal of investigating DNA polymorphisms of the CAST, TG, SPP1, MSTN and CAPN1 genes. Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) was employed to genotypes. Restriction enzymes revealed polymorphisms with 2 alleles and three genotypes of each CAST, TG, SPP1 and CAPN1 genes were determined in all breeds, while the MSTN locus was found to be monomorphic. Deviation from Hardy-Weinberg equilibrium (HWE) was not observed in the five cattle breeds on SPP1 locus. Significant deviation was observed from HWE in the TG and AB breeds on TG locus. Except for the TG and SAR breeds, genotype frequencies were not consistent with the HWE on CAPN1 locus. Four breeds were found at Hardy-Weinberg equilibrium except AB breed for CAST locus. Consequently, this study has shown that genetic polymorphisms do not exist in the MSTN gene but do exist in the remaining four genes that were examined in five Turkish native cattle breeds.

**Keywords:** Cattle, CAST, TG, SPP1, MSTN, CAPN1

## Türkiye'deki Beş Yerli Sığır Irkında Et Verim Özellikleri ile İlişkili Beş Genin Polimorfizmi

### Özet

Et verimi ile ilişkili olduğu bilinen birçok potansiyel gen vardır. Bu genler içerisinde yer alan; Calpain (CAPN1), Myostatin (MSTN), Calpastatin (CAST), Osteopontin (SPP1) ve Thyroglobulin (TG) genleri seleksiyonda et kalitesi ve verimini geliştirebilmek için kullanılabilir. Bu çalışmada, Zavot (n=60), Yerli Kara (n=59, YK), Güney Anadolu Kırmızısı (n=53, GAK), Boz Irk (n=60, BI) ve Doğu Anadolu Kırmızısı (n=49, DAK) ırkı sığırlarda; CAST, TG, SPP1, MSTN ve CAPN1 genlerinin DNA polimorfizmleri değerlendirilmiştir. Genotiplerin belirlenmesi için polimeraz zincir reaksiyonu ve restriksiyon parça uzunluk polimorfizmi (PZR-RFLP) kullanılmıştır. Restriksiyon enzimleri ile CAST, TG, SPP1 ve CAPN1 genlerinin her birinde 3 genotip ve 2 allel belirlenmiştir. MSTN geni ise monomorfik olarak bulunmuştur. SPP1 lokusunda beş sığır ırkında Hardy-Weinberg dengesinden (HWD) sapma gözlemlenmemiştir. TG lokusunda, Boz ve Yerli Kara ırklarında HW dengesinden önemli sapma gözlemlenmiştir. Boz ve Güney Anadolu Kırmızısı ırkları dışındaki ırklarda CAPN1 lokusunda HW dengesinden sapma olduğu belirlenmiştir. CAST lokusunun Yerli Kara dışında kalan dört ırkta HW dengesinde olduğu bulunmuştur. Sonuç olarak; bu çalışmada incelenen beş yerli sığır ırkında MSTN geni hariç, diğer dört gende polimorfizm olduğu belirlenmiştir.

**Anahtar sözcükler:** Sığır, CAST, TG, SPP1, MSTN, CAPN1

### INTRODUCTION

Molecular genetics techniques make it possible to describe genetic variation at different loci and to investigate the relationships between productivity and the variation

at a Quantitative Trait Locus (QTL). The ultimate goal in selection is to estimate an animal's genetic worth with greater accuracy, thereby increasing the genetic gain achieved through selection. For example, studies have reported that variations in genes that affect physiological



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events associated with the phenotype can affect quantitative variations in the phenotype <sup>[1]</sup>. Contemporary molecular genetics technology makes it possible to employ genetic markers or traits which exhibit a high correlation with the traits being researched in order to identify very productive animals. These genetic markers can also help to identify these traits when the animal is young, regardless of its gender. The rate of genetic progress using traditional selection methods is quite slow for animals such as cattle, sheep and goats, which have a long generation interval. Marker assisted selection (MAS) used together with traditional selection methods can be very effective for complex traits because it shortens the generation interval, accelerates the genetic progress and achieves improvement in the right direction <sup>[1,2]</sup>. Recently there has been a significant increase in the number of studies done in Turkey on polymorphism in genes that affect the economic traits of cattle breeds <sup>[3-6]</sup>. One of the most important economic traits of farm animals is meat production. There are many potential genes that are known to be related to meat production and that can be used for selection. Potential genes that can be used for selection to improve meat quality and production include Calpain (CAPN1), Myostatin (MSTN), Calpastatin (CAST), Osteopontin (SPP1) and Thyroglobulin (TG). Calpain activity is a cytoplasmic cysteine protease that requires the presence of calcium <sup>[7]</sup>. Suzuki and Sorimachi <sup>[8]</sup> identified two genes for calpain (CAPN1 [ $\mu$ -calpain] and CAPN2 [ $m$ -calpain]). Prior studies have identified a correlation between the regulation of CAPN1 activity and variations in meat tenderness as well as a quantitative trait locus on chromosome 29 where CAPN1 is located which affects meat tenderness <sup>[9]</sup>. Myostatin, which is also known as Growth Differentiation Factor-8 (GDF-8), is a member of the Transforming Growth Factor-B (TGF-B) family. GDF-8 is primarily synthesized in developing skeletal muscles and plays a role in skeletal muscle growth and differentiation. Muscular hypertrophy, a heritable trait commonly known as 'double muscling', is defined as excessive development of muscle mass <sup>[10,11]</sup>. Mutations occurring in the DNA sequences that encode the myostatin gene cause an increase in the muscle mass that is referred to as double muscling, which occurs due to inactivation of the myostatin gene. Senger et al. <sup>[12]</sup> were the first to describe osteopontin (OPN) as 60-kDa transformation-specific phosphoprotein that is secreted and that mediates cell matrix interactions and cellular signals by binding with integrin and CD44 receptors. Denhardt and Guo <sup>[13]</sup> reported that this protein is expressed in a number of different tissues. For this reason, the term 'secreted phosphoprotein 1' (SPP1) was proposed to reflect the factor's extensive functional role. The bovine SPP1 gene is made up of 7 exons that span about 7 kb of the genomic DNA. Schnabel et al. <sup>[14]</sup> and Khatib et al. <sup>[15]</sup> have identified the SPP1 gene on bovine chromosome 6 as a candidate for influencing milk and beef production traits in dairy cattle. Thyroglobulin (TG), which is a glycoprotein precursor of the thyroid hormones T3 and T4, is

only synthesized in the thyroid gland. The SPP1 gene is located at the centromere of chromosome 14 and contains 37 exons. Calpastatin (CAST) specifically inhibits the calcium-dependent neutral protease  $\mu$ -calpain, which is found in the tissue of mammals. Not only does calpastatin inhibit  $\mu$ - and  $m$ -calpain activity, it also regulates post-mortem proteolysis. Koohmaraie <sup>[16]</sup> identified an association between increased post-mortem calpastatin activity and reduced meat tenderness.  $\mu$ -calpain (CAPN1) and  $m$ -calpain (CAPN2) and their inhibitor calpastatin (CAST) are three enzymes that have a significant influence on this process.

The Anatolian Black (AB) breed of cattle is raised in the central Anatolian region. This breed has the smallest body size of all the Turkish native cattle breeds. However, this breed and its crossbreeds, together with the East Anatolian Red (EAR) breed, is the most important source of beef in Turkey. The Turkish Gray (TG) breed is raised in the regions of Marmara and Thrace. Similar breeds are bred in Ukraine, Romania, Hungary and Italy. The South Anatolian Red (SAR) breed is raised in southern Anatolia. It is bred for high milk yield and yields more milk than other Turkish native cattle breeds. The Zavot cattle breed was developed by crossbreeding the Simmental, Brown Swiss and EAR cattle breeds. The Zavot breed yields more milk and meat than EAR cattle, which is another cattle breed native to Turkey.

The number of genetically pure individuals from these native cattle breeds has been greatly reduced because of uncontrolled mating to improve yields, unconscious breeders, not exercising timely protection, and migration from rural to urban centers. Consequently, these breeds are faced with the threat of extinction. In order to survive, these breeds must become more productive. Yield traits related to the use of genetic markers is a good option to achieve this end. However, primarily genetic characterizations of known markers for these breeds are needed.

In the present study, the restriction fragment length polymorphism for the polymerase chain reaction (PCR-RFLP) technique was used to detect genetic polymorphism within five genes associated with meat production traits; CAST, TG, SPP1, MSTN and CAPN1 in EAR, SAR, TG, AB and Zavot cattle.

## MATERIAL and METHODS

### Samples and DNA Isolation

A total of 281 blood samples were collected from Zavot (n=60, Ardahan), AB (n=59, Ankara and Çankırı), SAR (n=53, Şanlı Urfa and Adana), TG (n=60, Edirne and Balıkesir) and EAR (n=49, Erzurum and Kars) cattle breeds. Genomic DNA was isolated with the phenol chloroform isoamylalcohol (25:24:1) method and examined using a NanoDrop 2000 (Thermo Scientific) for quantity and quality control.

### Polymorphism Detection and Genotyping

Table 1 shows the primer sequences used in amplification the relevant gene regions of the DNA that were obtained as well as the primer annealing temperatures, MgCl<sub>2</sub> concentrations and the restriction enzymes used to cut the DNA. All PCR reactions were performed on an Amplitronyx Series 6 thermal cycler. The PCR amplification reaction mixtures were performed in a total volume of 25 µl including ddH<sub>2</sub>O, 1X buffer, MgCl<sub>2</sub> (Table 1), dNTP (200 µM), primers (5 pmol) and Taq DNA polymerase (1 U/µL). This mixture was aliquoted into test tubes with approximately 100 ng DNA. The PCR reactions were cycled with routine procedures (denaturation, annealing and elongation). The annealing temperature for each primer was optimized (Table 1). The PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide. The PCR products of five genes were digested with fast digest enzymes (Table 2) according to the manufacturers' instructions (Fermentas, Vilnius, Lithuania). Restriction products were electrophoresed on 3% (CAPN1, TG, SPP1, CAST) and 5% (MSTN) agarose gel, and then displayed under a UV-transilluminator.

### Statistical Analysis

Direct counting was used to estimate genotype and

in Table 3. Digestion of the 290 bp fragment of the SPP1 gene with the *BsrI* restriction enzyme revealed a polymorphism with two alleles (Table 3). Three genotypes, CC (200 bp/90 bp), TT (290 bp) and CT (290 bp/200 bp/90 bp), were found in the SPP1 gene (Fig. 2). Results showed that the (T) allele had higher frequency in all breeds (Table 3). More animals had the TT genotype than any other genotype. Deviation from HWE was not observed in the five cattle breeds (Table 3).

Heterozygosity of the SPP1 locus varied from 0.17 to 0.37 in the five Turkish native cattle breeds. The heterozygosity, allele and genotype frequencies in these five Turkish native cattle breeds have been shown in Table 3.

### TG Locus

The allele and genotype frequencies of the TG locus obtained for five different cattle breeds have been shown in the Table 4. Digestion of the 545 bp fragment of the TG gene with the *PvuI* restriction enzyme revealed a polymorphism with two alleles (Table 4). Three genotypes, TT (545 bp), CC (370 bp/175 bp) and CT (545 bp/370 bp/175 bp), were found in the TG gene (Fig. 3). Results showed that the (C) allele had higher frequency in all breeds (Table 4). More animals had the CC genotype than any other

| Gene  | Primer   | PCR   | MgCl <sub>2</sub> | Ta     |
|-------|--|-------|-------------------|--------|
| CAPN1 | 5'-TTCAGGCCAATCTCCCCGACG-3'<br>5'-GATGTTGAACTCCACCAGGCCAG-3'                     | 670bp | 1mM               | 58.4°C |
| MSTN  | 5'-CCAATTACTGCTCTGGAGGAT-3'<br>5'-GGAGACATCTTTGTAGGAGTACAGC-3'                   | 124bp | 1.5mM             | 56.8°C |
| TG    | 5'-GGGGATGACTACGAGTATGACTG-3'<br>5'-GTGAAAATCTTGTGGAGGCTGTA-3'                   | 545bp | 1.5mM             | 60.7°C |
| SPP1  | 5'-GCAAATCAGAAGTGTGATAGA C-3'<br>5'-CCAAGCCAAACGTATGAGTT-3'                      | 290bp | 2mM               | 60°C   |
| CAST  | 5'-CCTCGACTGCGTACCAATTCCGAAGTAAAGCCAAAGGAACA-3'<br>5'-ATTCTCTGATGGTGGCTGCTACT-3' | 523bp | 2mM               | 55.4°C |

allele frequencies of genetic variants of the CAST, TG, SPP1, MSTN and CAPN1 genes. A chi-square statistic was used to check whether the populations were in Hardy-Weinberg equilibrium. PopGene32 software was used [17].

## RESULTS

### MSTN Locus

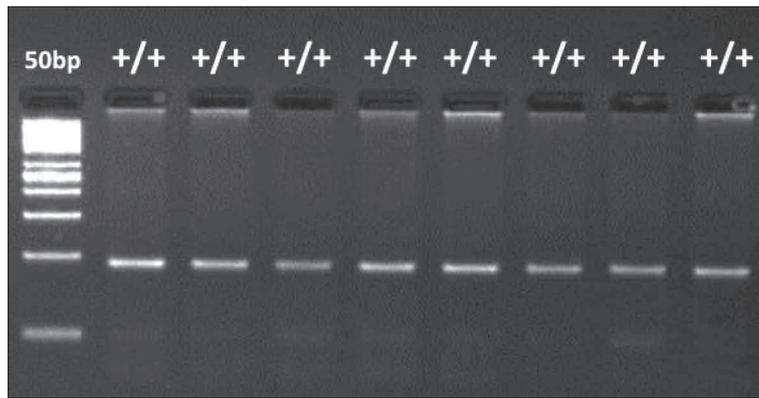
The MSTN locus was found to be monomorphic, and all cattle breeds examined were of the +/+ (100/24bp) genotype (Fig. 1).

### SPP1 Locus

The allele and genotype frequencies of the SPP1 locus obtained for five different cattle breeds have been shown

Table 2. Restriction endonucleases and restriction profiles  
Tablo 2. Restriksiyon endonükleazlar ve restriksiyon profilleri

| Gene  | RE                   | Restriction Profiles (bp) |             |         |
|-------|----------------------|---------------------------|-------------|---------|
| CAPN1 | <i>FokI</i>          | TT                        | CT          | CC      |
|       |                      | 670                       | 670/530/140 | 530/140 |
| MSTN  | <i>BstF5I (FokI)</i> | -/-                       | +/-         | +/+     |
|       |                      | 124                       | 124/100/24  | 100/24  |
| TG    | <i>PvuI</i>          | TT                        | CT          | CC      |
|       |                      | 545                       | 545/370/175 | 370/175 |
| SPP1  | <i>BsrI</i>          | TT                        | CT          | CC      |
|       |                      | 290                       | 290/200/90  | 200/90  |
| CAST  | <i>RsaI</i>          | CC                        | CG          | GG      |
|       |                      | 523                       | 523/266/257 | 266/257 |



**Fig 1.** MSTN genotyping after digestion with *FokI* restriction enzyme

**Şekil 1.** *FokI* restriksiyon enzimi ile kesilen MSTN genotiplendirmesi

**Table 3.** Heterozygosity, allelic genotypes frequency of SPP1 alleles C, T in five cattle breeds in Turkey

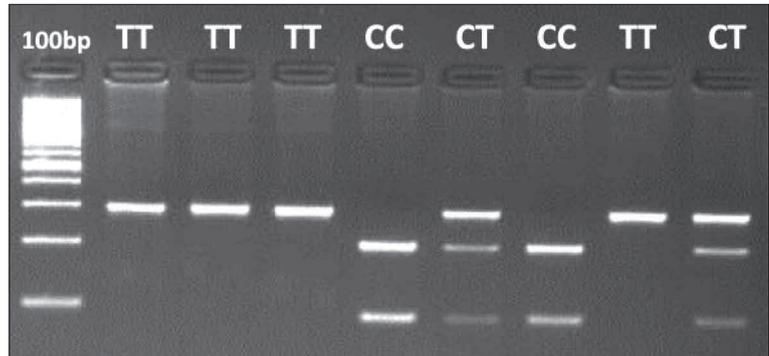
**Tablo 3.** Türkiye'deki 5 sığır ırkında SPP1 geninin C, T allelik genotip frekansları, heterozigotlukları

| Gene | Breed | n  | Allele Frequency |      | Genotype Frequency (%) |      |      | Heterozygosity |      | $\chi^2$ (df=1)    |
|------|-------|----|------------------|------|------------------------|------|------|----------------|------|--------------------|
|      |       |    | C                | T    | CC                     | CT   | TT   | Ho             | He   |                    |
| SPP1 | TG    | 60 | 0.17             | 0.83 | 3.3                    | 26.7 | 70.0 | 0.27           | 0.28 | 0.14 <sup>NS</sup> |
|      | AB    | 59 | 0.20             | 0.80 | 3.4                    | 33.9 | 62.7 | 0.34           | 0.33 | 0.08 <sup>NS</sup> |
|      | Zavot | 60 | 0.21             | 0.79 | 8.3                    | 25.0 | 66.7 | 0.25           | 0.33 | 3.83 <sup>NS</sup> |
|      | EAR   | 49 | 0.24             | 0.76 | 8.2                    | 32.7 | 59.1 | 0.33           | 0.37 | 0.81 <sup>NS</sup> |
|      | SAR   | 53 | 0.09             | 0.91 | 1.9                    | 15.1 | 83.0 | 0.15           | 0.17 | 0.91 <sup>NS</sup> |

NS: nonsignificant

**Fig 2.** SPP1 genotyping after digestion with *BsrI* restriction enzyme

**Şekil 2.** *BsrI* restriksiyon enzimi ile kesilen SPP1 genotiplendirmesi



**Table 4.** Heterozygosity, allelic genotypes frequency of TG alleles C, T in five cattle breeds in Turkey

**Tablo 4.** Türkiye'deki 5 sığır ırkında TG geninin C, T allelik genotip frekansları, heterozigotlukları

| Gene | Breed | n  | Allele Frequency |      | Genotype Frequency (%) |      |     | Heterozygosity |      | $\chi^2$ (df=1)    |
|------|-------|----|------------------|------|------------------------|------|-----|----------------|------|--------------------|
|      |       |    | C                | T    | CC                     | CT   | TT  | Ho             | He   |                    |
| TG   | TG    | 60 | 0.84             | 0.16 | 75.0                   | 18.3 | 6.7 | 0.18           | 0.27 | 6.36*              |
|      | AB    | 59 | 0.86             | 0.14 | 78.0                   | 16.9 | 5.1 | 0.17           | 0.24 | 5.01*              |
|      | Zavot | 60 | 0.80             | 0.20 | 61.7                   | 36.7 | 1.6 | 0.37           | 0.32 | 1.16 <sup>NS</sup> |
|      | EAR   | 49 | 0.77             | 0.23 | 61.2                   | 30.6 | 8.2 | 0.31           | 0.36 | 1.25 <sup>NS</sup> |
|      | SAR   | 53 | 0.91             | 0.09 | 83.0                   | 15.1 | 1.9 | 0.15           | 0.17 | 0.91 <sup>NS</sup> |

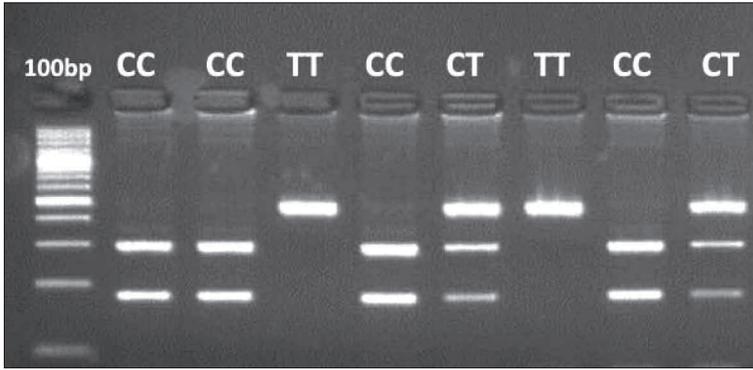
\* ( $P < 0.05$ ); NS: nonsignificant

genotype. Significant deviation was observed from HWE in the TG and AB breeds (Table 4).

#### CAPN1 Locus

Digestion of the 670bp fragment of the CAPN1 gene

with the *FokI* restriction enzyme revealed a polymorphism with two alleles (Table 5). The CAPN1 gene had three genotypes: TT (670 bp), CT (670 bp/530 bp/140 bp) and CC (530 bp/140 bp) (Fig. 4). Except for the TG and SAR breeds, genotype frequencies were not consistent with the HWE



**Fig 3.** TG genotyping after digestion with *PstI* restriction enzyme

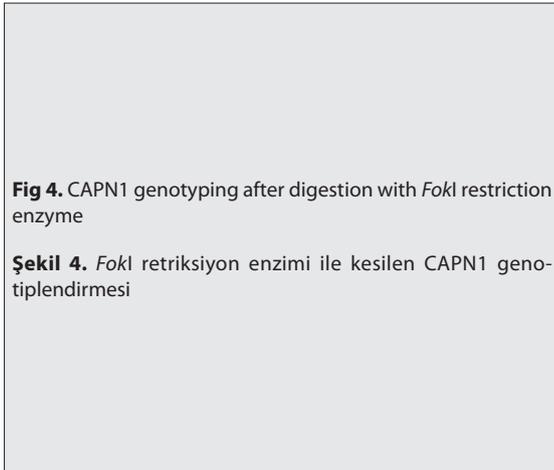
**Şekil 3.** *PstI* restriksiyon enzimi ile kesilen TG genotiplendirmesi

**Table 5.** Heterozygosity, allelic genotypes frequency of CAPN1 alleles C, T in five cattle breeds in Turkey

**Tablo 5.** Türkiye'deki 5 sığır ırkında CAPN1 geninin C, T allelik genotip frekansları, heterozigotlukları

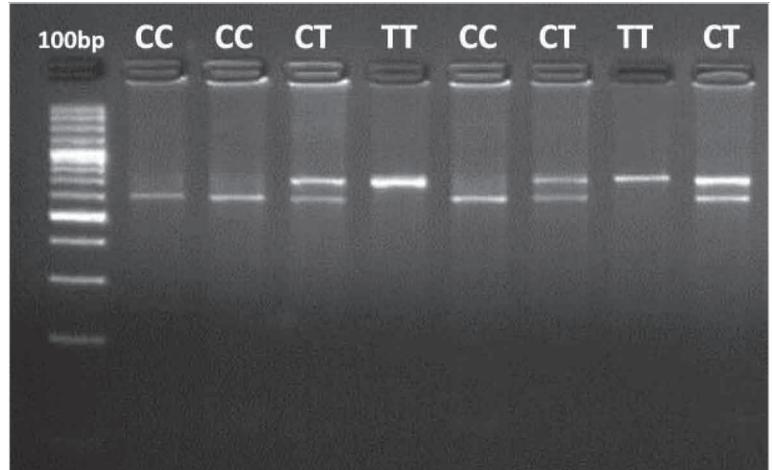
| Gene  | Breed | n  | Allele Frequency |      | Genotype Frequency (%) |      |      | Heterozygosity |      | $\chi^2$ (df=1)    |
|-------|-------|----|------------------|------|------------------------|------|------|----------------|------|--------------------|
|       |       |    | C                | T    | CC                     | CT   | TT   | Ho             | He   |                    |
| CAPN1 | TG    | 60 | 0.77             | 0.23 | 61.7                   | 30.0 | 8.3  | 0.30           | 0.36 | 1.76 <sup>NS</sup> |
|       | AB    | 59 | 0.75             | 0.25 | 66.2                   | 16.9 | 16.9 | 0.17           | 0.38 | 18.82*             |
|       | Zavot | 60 | 0.74             | 0.26 | 63.3                   | 21.7 | 15.0 | 0.22           | 0.39 | 11.89*             |
|       | EAR   | 49 | 0.75             | 0.25 | 61.2                   | 26.5 | 12.3 | 0.26           | 0.38 | 4.84*              |
|       | SAR   | 53 | 0.87             | 0.13 | 75.5                   | 22.6 | 1.9  | 0.23           | 0.23 | 0.03 <sup>NS</sup> |

\* ( $P < 0.05$ ); NS: nonsignificant



**Fig 4.** CAPN1 genotyping after digestion with *FokI* restriction enzyme

**Şekil 4.** *FokI* restriksiyon enzimi ile kesilen CAPN1 genotiplendirmesi

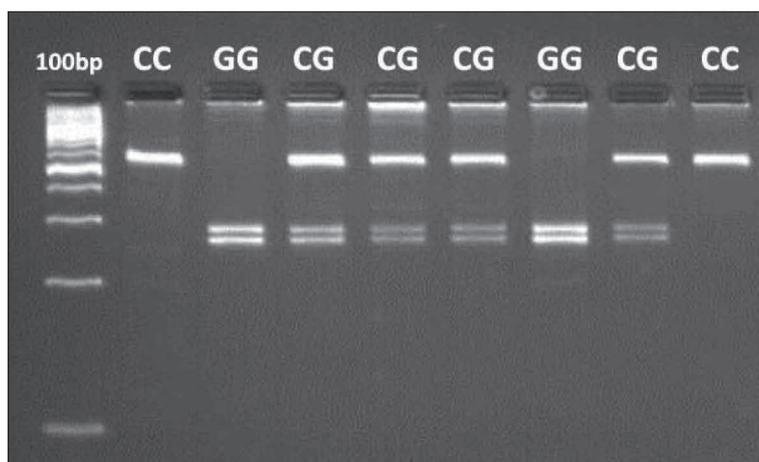


**Table 6.** Heterozygosity, allelic genotypes frequency of CAST alleles C, T in five cattle breeds in Turkey

**Tablo 6.** Türkiye'deki 5 sığır ırkında CAST geninin C, T allelik genotip frekansları, heterozigotlukları

| Gene | Breed | n  | Allele Frequency |      | Genotype Frequency (%) |      |      | Heterozygosity |      | $\chi^2$ (df=1)    |
|------|-------|----|------------------|------|------------------------|------|------|----------------|------|--------------------|
|      |       |    | C                | G    | CC                     | GC   | GG   | Ho             | He   |                    |
| CAST | TG    | 60 | 0.60             | 0.40 | 35.0                   | 50.0 | 15.0 | 0.50           | 0.48 | 0.07 <sup>NS</sup> |
|      | AB    | 59 | 0.67             | 0.33 | 38.9                   | 56.0 | 5.1  | 0.56           | 0.45 | 3.86*              |
|      | Zavot | 60 | 0.68             | 0.32 | 46.7                   | 43.3 | 10.0 | 0.43           | 0.44 | 0.01 <sup>NS</sup> |
|      | EAR   | 49 | 0.61             | 0.39 | 38.8                   | 44.9 | 16.3 | 0.45           | 0.48 | 0.21 <sup>NS</sup> |
|      | SAR   | 53 | 0.66             | 0.34 | 47.2                   | 37.7 | 15.1 | 0.38           | 0.45 | 1.51 <sup>NS</sup> |

\* ( $P < 0.05$ ); NS: nonsignificant



**Fig 5.** CAST genotyping after digestion with *RsaI* restriction enzyme

**Şekil 5.** *RsaI* restriksiyon enzimi ile kesilen CAST genotiplendirilmesi

( $P < 0.05$ ). The frequency of the (C) allele was found to be higher than the (T) allele (Table 5).

### CAST Locus

The 523bp fragment of the CAST gene was digested with the *RsaI* restriction enzyme and a polymorphism with two alleles was detected. The CAST gene had three genotypes: CC (523 bp), CG (523 bp/266 bp/257 bp) and GG (266 bp/257 bp) (Fig. 5). Except for the AB breed, genotype frequencies were consistent with the HWE ( $P < 0.05$ ) (Table 6).

## DISCUSSION

In the last 50 years, the world has experienced very rapid population growth. In addition, the Food and Agriculture Organization of the United Nations reported that food demand will double around the world in the next 50 years. Moreover, it has been predicted that global meat consumption will increase 68% and global milk consumption will increase 57% by 2030 [18]. Outside Europe and North America, in countries like Turkey that have a large population land area, the demand for meat cannot be met only by European breeds such as the Holstein. Therefore, it is important to improve the productivity of native breeds. To this end, the use of molecular markers associated with meat and milk production provides new opportunities to improve native breeds.

In animal breeding, some genes have been proposed as potential candidates with respect to traits that enhance economic value, such as milk yield, meat productivity traits and beef tenderness [19]. In the present study, the genetic polymorphisms of the CAPN1, MSTN, TG, SPP1 and CAST genes were evaluated in the TG, AB, Zavot, EAR and SAR cattle breeds. We used PCR-RFLP analyses to identify the different genotypes of each gene in these five Turkish native cattle breeds.

The frequency of the CAPN1-T allele was found to be higher than the frequency of the C allele in the Nellore

breed (*Bos indicus*) [20,21] and its crossbreeds, where the different breeds originated from *Bos taurus* [20]. Additionally, the CC genotype was not found or was found to be lower than other genotypes in breeds that originated from Zebu [20,21]. Similarly, in purebred Brahmans (*Bos indicus*), the TT genotype was found to be most frequent, while the CC genotype was not found at all [22]. On the other hand, the T allele was found to be more frequent in beef cattle breeds such as Red Angus, Charolaise and Limousin breeds [23]. At the same time, in a study that examined more animals, the frequency of the C allele (0.577) was found to be higher than that of the T allele (0.423) in a population that consisted of *Bos Taurus* (Hereford, Angus, Red Angus, Limousin, Charolais, Gelbvieh and Simmental). In the same study, the frequency of the C allele (0.64) was found to be higher than that of the T allele (0.36) in a population that consisted of *Bos taurus* and *Bos indicus* (Hereford, Angus, Brangus, Beefmaster, Bonsmara, and Romosinuano) [22]. In the present study, the C allele was found to be more frequent than the T allele in five Turkish local cattle breeds when compared with the same European cattle breeds which originate from *Bos Taurus*. Furthermore, the CC genotype was found to be more frequent than other genotypes, while the TT genotype was found to be less frequent in five Turkish local cattle breeds. Therefore, it is thought that the TT genotype could be used to improve meat yield and flavour in Turkish local cattle breeds. To this end, the TT genotype frequency could be increased in Turkish local cattle breeds because the TT genotype is associated with favourable meat production traits that are important [22,24]. Furthermore, the CT and TT genotypes were associated with the best meat tenderness when compared with the CC genotype [24,25]. Despite the fact that the TT genotype frequency is low in Turkish local cattle breeds, this genotype was found to be have the lowest frequency in the SAR breed (0.019). This cattle breed is bred for milk and has a higher milk yield than that of other Turkish native cattle breeds. The second lowest genotype frequency was found in the TG breed (0.083), and in terms of milk yield, this breeds ranks second in Turkish native cattle breeds. Consistent with these

findings, the C allele was found to be higher in dairy cattle breeds such as Simmental, Black-and-White and Polish Red cattle breeds [23].

It has been determined that a mutation in the MSTN gene was associated with the double-muscle phenotype in the Piemontese cattle breed [26]. Subsequently, this mutation was found in the Belgian Blue [27] and South Devon [28] cattle breeds. However, this mutation was not reported in other cattle breeds including Turkish native cattle breeds [5]. This mutation was not found in the five Turkish native cattle breeds that were examined in this study. This provides strong evidence that this mutation is not present in Turkish native cattle breeds. Despite the fact that these mutations are associated with high carcass weight, they were found to be associated with increased calving difficulty and reduced meat flavour [29]. Therefore, this situation can be considered to be good for Turkish native cattle breeds, and leads to the conclusion that these cattle breeds have maintained their purity.

The TG gene in cattle was found to be not associated with the traits of daily weight gain, weight at slaughter, rib eye fat and rib eye area [30]. However, the TG genotypes were associated with the marbling score in beef cattle breeds such as Aberdeen Angus, Shorthorn and Waghu cattle [31], and the T allele was found to be associated with a higher marbling score [32]. On the other hand, the TT genotype of this gene was found to be associated with the thickness of back fat in beef cattle [30]. Additionally, it was reported that the TT genotype of this gene may be used in marker assisted selection (MAS) for higher intramuscular fat content as a desirable trait [33,34]. Therefore, it is thought that the detection of TG gene polymorphism can be used for efforts to improve beef quality traits and for characterizing Turkish native cattle breeds.

The frequency of the TG-TT genotype was either not found or found to be very low in many cattle breeds such as Holstein, indigenous Polish Red, Hereford, Limousin [35,36], Charolais, Simmental, Angus, Belgian Blue [36], Red Angus [34], indigenous Japan Wagyu cattle [37] and Korean native cattle [38]. The current study also found the TG-T allele frequency in five Turkish native cattle breeds to be lower than the C allele frequency. In addition, the TT genotype frequency was found to be lower than the frequency of other genotypes in the five Turkish native cattle breeds evaluated in this study. This study and other studies suggest that the TG-C allele can be considered to be predominant in cattle.

Osteopontin (OPN, also called secreted phosphoprotein 1 or SPP1) plays a role in different processes such as the regulation of growth and development of the fetus, initiation and maintenance of pregnancy, and proliferation and differentiation of the mammary gland in mammals [39,40]. Because it has so many tasks, it is thought that the OPN

gene could be used as a marker gene for milk performance traits [35], and growth [41]. It has been reported that the OPN (SPP1)-C allele has a positive effect on milk fat yield and milk protein content, while others report that it has a negative impact on milk yield [42]. In general, the frequency of the OPN (SPP1)-T allele was found to be higher than that of the C allele in different cattle breeds such as the Jersey [43], Holstein, Polish Red, Hereford and Limousin breeds [35]. In a study that examined EAR and SAR breeds, the T allele frequency was found to be higher than that of the C allele [44]. Similarly, the present study also found that the T allele frequency was higher than that of the C allele in the five Turkish native cattle breeds that were examined. Even in the SAR breed, which is known to be the most prevalent dairy breed of Turkish native cattle breeds, this rate was found to be over ninety percent.

The CAST gene has been associated with the trait of meat tenderness [45] and this gene has been suggested as a marker for tenderness [21,46]. The CAST-CC genotype has been found to be a desirable genotype for tenderness in many cattle breeds such as the Brahman, beef cattle crossbreeds [22,45], Nellore [21], Hanwoo (Korean cattle) [47], Red Angus, Charolais, Limousin, Simmental, Hereford, Polish Friesian and Polish Red [23]. Therefore, the CAST gene is important for work done to improve the quality of meat. The CAST-C allele frequency was found to be higher than that of the G allele in many cattle breeds which originated from *Bos taurus* [Hanwoo (Korean cattle) [47], Angus, Limousin, Charolais [45], Simmental, Hereford and Friesian [23]], *Bos indicus* [Nellore [21,48] and Brangus [48]], and *Bos taurus-Bos indicus* crossbreeds [Angus x Nellore, Rubia Gallega x Nellore and Canchim [48]]. In a previous study, the C allele frequency was found to be higher in the TG and TG x Brown Swiss crossbreeds [49]. Similarly, the CAST-C allele frequency was found to be higher in all Turkish native cattle breeds that were examined in the current study, as is the case with other cattle breeds. However, the CG genotype was found to be high, thus it is thought that the CAST gene could be used to improve the tenderness trait of Turkish native cattle breeds.

Many indigenous livestock breeds are still bred due to their adaptation traits and lower maintenance costs. However, the productivity of these breeds is lower than many European breeds. Despite this, the conservation and survival of the different genotypic variants in the genetic pool of a species is very important for genotyping livestock for selection against various infections, effects and environmental conditions. Further studies such as association analysis and QTL analysis are required to investigate the connection with productivity traits in native farm animal populations. In conclusion, this study has shown that genetic polymorphisms do not exist in the MSTN gene but do exist in the remaining four genes that were examined in five Turkish native cattle breeds.

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