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Research Article

The Use of Various SNPs in CAST and CAPN1 Genes to Determine the Meat Tenderness in Turkish Grey Cattle [1]

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Abstract

The aim of this study is to investigate the relationship between some genotypic characteristics of purebred Turkish Grey Cattle (TGC) and beef tenderness characteristics. There is a linear relationship between meat tenderness and the increasing calcium activity after slaughtering. Calpastatin (CAST) is a neutral protease inhibitor of Calpain (CAPN1) in mammalian tissues. The three polymorphic places in the CAST and the CAPN1 genes in cattle (*Uo*G-CAST, CAPN1 316 and 4751) are known as the markers of beef quality. The relationship between the tenderness traits of the *longissimus dorsi* (LD) and the 3 beef quality markers (3 SNPs) in pure TGC have been investigated and discussed. PCR-RFLP and ARMS-PCR methods were utilized to identify the genotypes. In order to determine the water holding capacity (WHC), cooking loss (CL) and the shear force (SF), samples extracted from LD were probed. The average and standard error SF of a two-year-old TCG bred in extensive conditions was 4.339±0.217 kg/cm^2 for the heifers and 4.689±0.569 kg/cm^2 for bulls. Both alleles of the *Uo*G-CAST (C/G), the CAPN1 316 (C/G) and the CAPN1 4751(C/T) polymorphisms in the samples were observed. The average SF of 3.943±0.441 kg/cm^2, 4.537±1.666 kg/cm^2, and 3.869±0.721 kg/cm^2 were used for the CAST-CC, the CAPN1 4751-CC and the CAPN1 316-GC, respectively in order to cut the muscle fibers of the genotypes that have a positive effect on tenderness. No cattle of the CAPN1 316-CC genotype was identified among the samples. The average and standart error WHC, CL and SF values for the entire sample including heifers and bulls were found as 11.693±0.761%, 26.952±0.636%, and 4.483±0.252 kg/cm^2, respectively. In conclusion, the presence of genetic variation in specific SNP markers of beef tenderness in purebred TGC can contribute to the process of raising TGC with more tender meat.

Keywords: *Beef texture, Water Holding Capacity, Cooking Loss, CAST, CAPN1, PCR-RFLP, ARMS-PCR*

Boz Irk Sığırın Et Gevrekliğini Belirlemede CAST ve CAPN1 Genlerindeki Kimi SNP'lerin Kullanımı

Özet

Bu çalışmanın amaçı saf Boz ırk sığırın (TGC) kimi genotipik özellikleri ile et gevreklik özelliği arasındaki ilişkiyi araştırmaktır. Kesim sonrası sığır etlerinde artan kalsiyum aktivitesi ile et gevrekliği arasında doğrusal bir ilişki vardır. Kalpastatin (CAST), memeli dokularında bulunan kalpain'in (CAPN1) nötr bir proteaz inhibitörüdür. Sığır CAST ve CAPN1 genlerinde üç polimorfik yer (*Uo*G-CAST, CAPN1 316 ve 4751) et kalite markörü olarak bilinir. Saf TGC'ın *longissimus dorsi* (LD) kası gevreklik özellikleri ile 3 et kalite markörünün ilişkileri araştırılıp tartışılmıştır. Genotipleri tanımlamada PCR-RFLP ve ARMS-PCR metotları kullanılmıştır. Etin su tutma kapasitesi (WHC), pişme kaybı (CL) ve tekstürünü (SF) belirlemek için LD kası örnekleri üzerinde çalışılmıştır. Extansif koşullarda yetiştirilen iki yaşındaki TGC sığırların SF ortalama ve standart hataları düveler de 4.339±0.217 kg/cm^2, erkekler de 4.689±0.569 kg/cm^2 olduğu saptanmıştır. Çalışılan et örneklerinde *Uo*G-CAST (C/G), CAPN1 316 (C/G) ve CAPN1 4751(C/T) polimorfizmlerinin her iki alleli de gözlenmiştir. Et gevrekliği üzerine olumlu etkisi olan genotiplerin, kas liflerini kesmek için ortalama CAST-CC için 3.943±0.441 kg/cm^2, CAPN1 4751-CC için 4.537±1.666 kg/cm^2 ve CAPN1 316-GC için 3.869±0.721 kg/cm^2 SF uygulanmıştır. Örneklerde CAPN1 316-CC genotipinde sığır belirlenmemiştir. Dişi ve erkek sığırlara ait tüm örneklerin dahil olduğu WHC, CL ve SF ortalama ve standart hata değerleri sırasıyla; %11.693±0.761, %26.952±0.636 ve 4.483±0.252 kg/cm^2 olarak belirlenmiştir. Sonuç olarak, safkan TGC da et gevrekliğine özgü SNP markörlerin genetik çeşitliliğin olması, daha gevrek etli TGC sığır yetiştirme sürecine katkıda bulunabilir.

Anahtar sözcükler: *Et tekstürü, Su Tutma Kapasitesi, Pişme Kaybı, CAST, CAPN1, PCR-RFLP, ARMS-PCR*

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INTRODUCTION

Meat is obtained from warm-blooded and healthy cattle, ovine and poultry by butchery. The part that is left after all the blood is drawn and the parts that are not suitable for consumption are removed is the meat on the bone. The word "meat" usually refers to the skeletal muscles, which is also called striated muscles. After the rigor mortis stage ends, the meat which has become rigid softens with the ripening when it is not sliced and becomes tender. The meat should be kept in a cool and hygienic environment to ease the effects of rigor mortis. During the ripening pH ranges between 5.2 and 6.2 in butchery animals as a result of various enzymatic activities. The meat with pH 6.4 is suspected to have bacterial decomposition. The tenderness of meat which has a huge impact on customer satisfaction is one of the most important issues in beef cattle production [1]. CAPN proteolytic system is responsible for the tenderizing process after death. CAST prevents μ- and *m*-Calpain activity and regulates postmortem proteolysis. There is a linear correlation between the calcium activity after death and the meat tenderness [2]. Many studies show that CAPN-CAST system is important to the normal development of skeletal muscles. Smith *et al*.[3] reported that the specific SNPs in CAST and CAPN1 loci are related to some genetic effects that are important to meat tenderness. Also, most reports of the same subject point out the relationship between the CAST and CAPN1 genetic polymorphism and LD and the shearing resistance of the muscle [4-6]. Instead of choosing the best meat after determining the meat quality parameters (taste, juiciness or tenderness), it would be wiser to choose the animals with the genotypes which have more qualified meat. It is possible to choose the animals with more qualified meat by determining the polymorphisms in CAST and CAPN1 gene loci of the animals without using complicated selection procedures. In *Bos Taurus* beef, there is a relationship between the SF shearing resistance and *Uo*G-CAST SNPs during the postmortem 7th, 14th and the 21st days. The cattle with *Uo*G-CAST CC genotype are likely to have more tender beef compared to the cattle with GG genotype, and the cattle with CG genotype have to medium tenderness beef [5-7]. In addition, some researchers who worked on *Bos taurus* [3,8-10] and *Bos indicus* [4,11,12] beef found a correlation between the SF values and the CAPN1 316 SNPs and 4751 SNPs on the 7th and 14th days after the slaughter ($P<0.05$). The SF applied to homozygous cattle meat with the CAPN1 316 CC genotype had 10% and 14.6% less resistance compared to the heterozygote CG genotypes and homozygous GG genotypes, respectively [13]. Beef from *Bos Taurus* cattle with the CAPN1 4751 CC genotypes are more likely to be tender compared to the cattle with other genotypes [8,14].

Some commercial genetic indicators have been developed to increase beef tenderness, such as the *Uo*G-CAST and the CAPN1 316 markers [6,14]. National Cattlemen's Beef Association in the USA has approved the efficiency of those genetic markers. The association recommends the cattlemen to increase the ratio of the CAPN1 316/4751 haplotype C/C frequency within the cattle population to have more tender beef [6].

The aim of this study is to obtain empirical data from the purebred TGC's beef (from the rib steaks) samples by comparing their WHC, CL, SF data to TGC with different genotypes which are formed by the *Uo*G-CAST (CAST/*Rsa*I), the CAPN1 316 and 4751 polymorphisms.

MATERIAL and METHODS

Animals

The samples that were extracted from 17 TGC (10 males and 7 females) from the rural cattle breeding barns in Enez District, Edirne in 2013 were used. The barns from which the samples were extracted utilized extensive breeding methods in grazing lands. In this study, female and male animals bred in Thrace and slaughtered at 24-months-old were used.

Separation and Evaluation Time of The LD

TGC brought from the rural grazing lands of Çandır village in Enez district were cut off in Keşan slaughterhouse. The LD samples of our research material were separated from TGC carcasses after 24 h of waiting in slaughterhouse.

The assessments of WHC (%), CL (%) and Warner-Bratzler SF (kg/cm^2) on the *LD* were done on the 21st day after death at Istanbul University, Faculty of Veterinary Medicine, Zootechnics Department Laboratory.

Calculation of The WHC

In order to measure the WHC "Passive Drip Loss" method [15] was used. To do this, 100 g samples were extracted from the LD muscle and weighted (weight1). The samples were placed in a polyethylene bag without touching the surface of the bag and they were weighed again after they were stored at +4ºC for 24 h (weight2). WHC was calculated with the following formula [15].

WHC (%) = [(weight1-weight2)/weight1]*100

Calculation of The CL

The meat frozen at -18ºC was thawed at +4ºC and 100 g meat samples were placed in polyethylene bags and vacuumed. The meat was cooked in water-bath for an hour until the internal temperature of the meat reached 80ºC. The cooked meat was cooled down for 12 h at +4ºC and the surface was dried before it was weighed. CL percentage was calculated with the following formula [15,16].

CL (%) = [(weight of raw steak after thawing – weight of cooked steak)/weight of raw steak after thawing] × 100.

Texture Analysis

After the calculation of CL, the cooked meat was sliced with a SF knife with a 50-kg cutting power at 20 cm/min speed to determine the shearing resistance of the muscle fibers for the texture analysis. The meat cooked at 70-72ºC were cooled down 5-6ºC and they were sliced as a section of 2.54 x 2.54 cm parallel to the muscle fibers. Six samples were extracted from each LD and they were sliced. *The highest force* (kg/cm^2) and *force* x *time* graphics obtained during the application of the SF knife for each sample were saved on the computer. The peak SF value for a muscle of an animal was calculated by averaging the values obtained from the six samples extracted from that animal [11,15].

DNA Isolation

Genomic DNA of the tissue samples taken from TGC were isolated using Fujifilm QuickGene-mini-80 device and tissue kit (Quick Gene DNA Tissue kit, Fujifilm, UK) based on the instructions given by the manufacturer. A nanodrop ($A^{260/280}$) was used to determine the amount of the DNA and the samples were stored at –20ºC in Trakya University Biotechnology and Genetics Laboratory.

Amplification of the Target DNA Samples and Identification of the Genotypes

The target DNA was amplified and The PCR-RFLP method with R*sa*I restriction enzyme was utilized to identify the genotypes of CAST gene SNP (AY008267.1:g.282C>G) according to the works of Schenkel *et al.*[5] and Curi *et al.*[11]. In order to determine the CAPN1 4751 SNP (AF 248054.2:g.6545C>T) and the CAPN1 316 SNP (AF252504. 2:g.5709C>G) genotypes, the ARMS-PCR method was used based on the work of Rincón and Medrano [17]. The primers (Sentegen Biotech, Ankara/Turkey) used in this study were given in *Table 1* and fragment sizes were amplified using the Bioneer My Genie 96 Thermal Block PCR device (Bioneer Corporation, South Korea). Amplification of DNA was run in the 2% horizontal gel electrophoresis and was used for the genotyping by "DNR BioImaging Systems Minibis Pro. Jerusalem, Israel".

Table 1. *Primary Sequences used in PCR and the product size obtained*

Marker	Primer Sequences (5′ - 3′)	bp
**UoG* CAST	[1]Fop:CTCGACTGCGTACCAATTCCGAAGTAAAGCC AAAGGAACA	523
	[2]Rop: ATTTCTCTGATGGTGGCTGCTCACT	
**CAPN1 316	[3]Fip: TTTCCTGCAGCTCCTCGGAGTGGAA**G**GG	269
	[4]Rip: GCTCCCGCATGTAAGGGTCCA**G**GG	228
	[1]Fop: GCTGTGCCCACCTACCAGCATC	446
	[2]Rop: CAGGTTGCAGATCTCCAGGCGG	
**CAPN1 4751	[3]Fip: GCATCCTCCCCTTGACTGGGGGGAAA**C**CC	158
	[4]Rip: GTCACTTGACACAGCCCTGCGCC**G**CA	231
	[1]Fop: CCTGGAGTCCTGCCGCAGCATGGTCAAC	334
	[2]Rop: AAGCTGCAGGAGCTGCCCAAAGCCAGGC	

** RFLP method, ** ARMS method, [1] **Fop:** Forward outer primer, [2] **Rop:** Reverse outer primer, [3] **Fip:** Forward inner primer, [4] **Rip:** Reverse inner primer, **bp:** Base pair*

Statistical Analysis

We genotyped TGC based on the polymorphic The *Uo*G-CAST loci in CAST gene and the CAPN1 316 and 4751 loci in CAPN1 gene. After genotyping, the samples underwent phenotypic analysis to calculate WHC%, CL% and SF (kg/cm^2). Averages (X), standard error (s_x), the genotypic and phenotypic correlations (r) were calculated using SPSS statistics program. In addition, two-tailed t-test was used to compare the results. According to the Hardy-Weinberg (HW) equilibrium, the fit test of the distribution of Allele and genotype frequencies was calculated by the Chi-square (χ^2) test. The distribution of genotypic frequencies was evaluated based on the 5% significance level of the Chi-square table.

RESULTS

Cattle with one combination of the two different patterns one of which is matched with either homozygous or heterozygote genotypes in the *Uo*G-CAST, the CAPN1 316 and 4751 loci were identified and the three unique band patterns of the cattle genotypes (except the CAPN1 316 CC genotype) were observed in the electrophoresis gel. The genotype and allele frequencies of the 3 different SNPs of the sample 17 purebred TGC were calculated in accordance with HW equilibrium, which are shown in *Table 2*.

Two genetic variances (C and G) were identified in The *Uo*G-CAST polymorphism structure of TGC samples. According to the pattern images formed on the electrophoresis gel; homozygous CC genotype, homozygous GG genotype; and heterozygotes-GC were characterized and defined to have one band with 523 bp, two bands with 257 and 266 bp and three bands with 523, 257 and 266 bp, respectively. In all of the TGC samples the frequency of the *Uo*G-CAST C allele, which is thought to have a positive effect on beef yield, is 0.559 and the distribution between C/G alleles is in HW equilibrium ($P>0.05$). The *Uo*G-CAST genotypic frequencies of the male samples were significant ($P<0.05$) and in male samples were found to have a higher frequency of C allele (0.714). The genotypic frequencies of CC, CG and GG in TGC were observed as 0.312, 0.493 and 0.195, respectively *(Table 2)*. TGC is in HW equilibrium for the *Uo*G-CAST SNP ($P>0.05$).

In the TGC, the CAPN1 316 polymorphism genotypes were defined based on the patterns they form on the electrophoresis gel with the use of ARMS-PCR method. The ones forming 3 bands with 228, 269 and 446 bp were defined as Heterozygote CG genotype while the ones

Table 2. *The UoG-CAST (Calpastatin), the CAPN1 (μ-Calpain) 316 and 4751 allele and genotype frequencies of TGC samples*

Cattle Race	Animal Number	*Uo*G-CAST Allele Frequency		*Uo*G-CAST Genotype Frequency (n)			*Chi-square*
Turkish Grey Cattle	17	C	G	CC	CG	GG	0.529
		0.559	0.441	0.312 (5)	0.493 (9)	0.195 (3)	
		CAPN1 316 Allele frequency*		CAPN1 316 Genotype frequency* (n)			
		C	G	CC	CG	GG	36.41*
		0.059	0.941	0.003 (0)	0.111 (2)	0.886 (15)	
		CAPN1 4751 Allele frequency		CAPN1 4751 Genotype frequency (n)			
		C	T	CC	CT	TT	1.582
		0.412	0.588	0.171 (2)	0.485 (10)	0.346 (5)	

** The population is not in HW equilibrium (P<0.05)*

Table 3. *The comparison of the results related to WHC %, CL % and Meat Texture (SF kg/cm²) of the LD samples based on Gender, the UoG-CAST, the CAPN1 4751 and 316 genotypes in all TGC*

Phenotypic Features	All Group	Sex Group		UoG-CAST Genotype		
		Female	Male	CC	GC	GG
SF(kg/cm²) (X±s_x)	4.483±0.252	4.339±0.217	4.689±0.569	3.943±0.441	4.488±0.298	5.55±0.633
CL % (X±s_x)	26.952±0.636	27.505±0.929	26.161±0.866	26.688±1.108	27.584±1.015	25.793±1.560
WHC % (X±s_x)	11.693±0.761	12.687±1.082	10.272±0.947	10.188±0.969	11.667±1.293	14.772±0.740
Phenotypic Features	**All Group**	**CAPN1 4751 Genotype**			**CAPN1 316 Genotype**	
		CC	CT	TT	GG	GC
SF(kg/cm²) (X±s_x)	4.483±0.252	4.221±0.873	4.537±1.666	4.986±1.303	4.565±1.102	3.869±0.721
CL % (X±s_x)	26.952±0.636	24.385±2.123	27.688±2.730	26.506±2.530	27.068±2.866	26.077±0.270
WHC % (X±s_x)	11.693±0.761	12.406±4.843	11.850±3.568	11.093±2.554	11.644±3.251	12.060±3.251

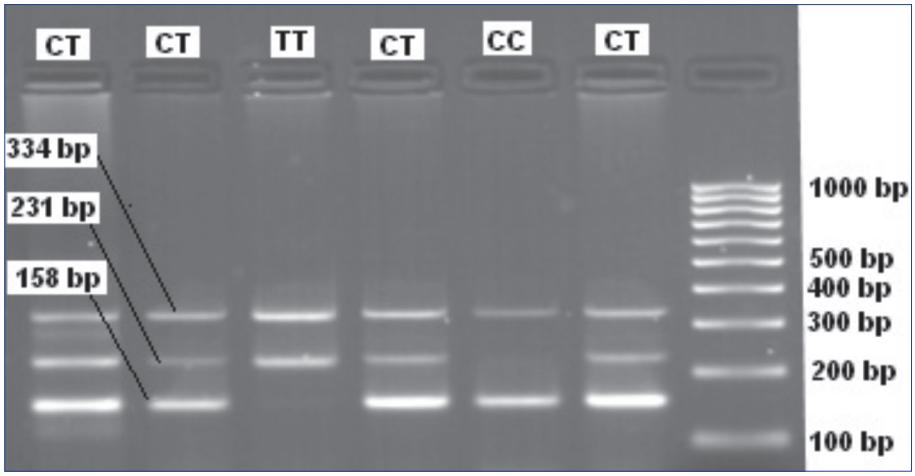

Fig 1. The agarose gel electrophoresis images of TGC with the CAPN1 4751 SNP genotypes using ARMS-PCR method

forming 2 bands with 269 and 446 bp long were defined as Homozygous GG genotype. However, there were no TGC carrying 2 bands with 228 and 446 bp that define the homozygous CC genotype in our sample. The CAPN1 316 C/G allele frequencies distribution of the samples is significant (P<0.05). There were no cattle with CC genotype in our TGC sample group and the C allele frequency which has a positive effect on the tenderness of the beef was quite low (0.059). TGC is not in HW equilibrium for the CAPN1 316 SNP (*P*<0.05) *(Table 2)*.

In the TGC, the CAPN1 4751 SNP with Homozygous CC genotype, Heterozygote CT genotype and Homozygous TT genotype were observed to form two band patterns with 334 and 158 bp, three band patterns with 334, 231 and 158 bp and two band patterns 334 and 231 bp size, respectively in the electrophoresis gel with the use of ARMS-PCR method *(Fig. 1)*. The distribution of the CAPN1 4751 C/T allele frequencies in TGC samples are in HW equilibrium. Similarly, the distribution of the CAPN1 4751 genotypic frequency was found to be in HW equilibrium (*P*>0.05).

Table 4. *The correlations derived for meat quality features and SNPs*

Traits	UoG CAST	CAPN1 4751	CAPN1 316	WHC (%)	CL (%)	SF (kg/cm²)
*Uo*G-CAST						
CAPN1 4751	0.155					
CAPN1 316	-0.052	0.400				
WHC (%)	-0.126	0.135	0.043			
CL (%)	0.015	-0.106	-0.122	0.263		
SF (kg/cm²)	-0.227	-0.221	-0.216	0.148	-0.199	
Gender	0.262	**0.536***	0.306	0.379	0.252	-0.166

** In bold, significant values (except diagonal) at the level of significance P<0.05 (two-tailed test)*

The ratio of heterozygous TGC was observed in higher frequency than other genotypes with the CAPN1 4751 *(Table 2)*.

WHC, CL and SF values for the LD samples of TGC genotypes with the UoG-CAST, the CAPN1 4751 and 316 are presented in *Table 3*. According to the results of the study, TGC with the *Uo*G-CAST CC (3.943±0.4414 kg/cm²) genotype have more tender meat compared to TGC CG and GG genotypes. Also the statistical relationship was found that between the SF value after death and the *Uo*G-CAST CC SNP genotype ($P<0.05$) of the LD muscle. The SF values of TGC with the CAPN1 316 GC genotype were found 3.869±0.7214 kg/cm². TGC with the CAPN1 316 GC genotypes were determined to have more tender meat than the GG genotypes (4.565±1.1025 kg/cm²). The difference between each other measurement was found statistically insignificant ($P>0.05$). According to the SF values of TGC with the CAPN1 4751 CC (4.221±0.8734 kg/cm²) in our sample, TGC with CC genotype were found to yield more tender meat compared to the cattle with CT and TT genotypes although the difference was not statistically significant ($P>0.374$).

The marker combinations associated with meat tenderness in TGC samples were compared. TGC with the toughest meat in our sample was the male cattle with GG/TT/GG genotype where the *Uo*G-CAST "GG", the CAPN1 4751 "TT" and the CAPN1 316 "GG" combinations merge with 6.557 kg/cm² SF value. On the other hand, the male cattle with CC/CT/GG genotype seemed to have the tenderest meat with 2.329 kg/cm² SF. The haplotype C/C/C frequency, which is positive for meat tenderness, in our TGC sample was found 0.014. The triple genotypic combinations affecting the meat tenderness in a positive way were not evaluated statistically in our sample due to their inadequate number in our sample. Except for the linear correlation between TGC with the CAPN1 4751 genotype and gender ($P<0.05$), the analysis results for the meat quality and SNP correlations did not yield a significant difference between SF, CL, WHC, gender, the *Uo*G-CAST, the CAPN1 316 and 4751 poly-morphisms *(Table 4)*.

DISCUSSION

The frequency of C allele in the *Uo*G-CAST locus is reported to be 0.74-0.79 in *Bos taurus* genetic groups [6], 0.69 [11], 0.72 [18] and 0.75 [19] in *Bos taurus* crossbreeds, 0.64 [20] in Aberdeen Angus cattle, 0.61 in Pirenaica cattle and 0.70 in Parda de Montana [21], 0.623 on average in Aberdeen Angus, Limousine, Charolais and Simmental breeds [5], 0.623 in Nellore-*Bos indicus* [11]. The C allele frequency in our sample which is made up of purebred TGC is 0.559, which is also lower than the frequencies reported by previous researchers [20,21]. For the *Uo*G-CAST SNP genotypes Gill *et al.*[22] found the CC, CG and GG genotypic frequencies in Aberdeen Angus cattle as 0.41, 0.47 and 0.12, respectively. On the other hand, Schenkel *et al.*[5] found them as 0.430, 0.398 and 0.172, respectively for *Bos taurus* while Curi *et al.*[11] found 0.377, 0.491 and 0.132, respectively for purebred *Bos indicus* Nellores. In our study, the ratio of the purebred TGC with CC genotype which expected to beef with more tenderness is lower than previous researches [20,21], except Kök *et al.*[23]. The CAPN1 316 C allele is associated with meat tenderness. TGC with the CAPN1 316 C allele frequency in our study is close to *Bos taurus* x *Bos indicus* crossbreeds [11] and similar to that of Pirenaica and Parda de Montana breeds. The frequency ratio of the TGC with the CAPN1 316 CC genotype is quite low. Except for Aberdeen Angus [22] and Hanwoo cattle [24], *Bos taurus* with CC genoype have not been observed in previous studies [11,25,26]. The CAPN1 4751 C allele frequency (0.412) that is considered to have a favourable impact on the beef tenderness seems to be higher than the other breeds except for Aberdeen Angus (0.65) [22] and Hanwoo cattle (0.80) [24]. TGC with the CAPN1 4751 CC genotype frequency (0.171) were found to be higher compared to Nellore (0.00) and Canchim (0.07) [4], Simmental and their crossbreeds (0.05) [26]. The ratio of TGC with CC genotype, which is favourable for meat tenderness, is lower than Brahman crossbreeds (0.53) [26], Aberdeen Angus (0.41) [22] and Hanwoo cattle (0.64) [24] populations.

Lee *et al.*[27] who worked on Hanwoo cattle found the average SF as 5.803±0.275 kg cm², for the heifers as 6.598±0.265 kg/cm², for the bulls as 7.200±0.275 kg/cm² and 3.400±0.061 kg/cm² for calves. Soysal [28] who worked on the texture of beef stated that gender and muscle factor do not seem to be statistically significant ($P>0.05$). When the result of the study is compared to Yüksel *et al.*[29] who worked on East Anatolian Red (EAR) breeds and found (6.84-9.46±0.49 kg/cm²) and to Özlütürk *et al.*[30] who found (5.76±0.58 kg/cm²), the meat obtained from TGC can be said to have higher levels of tenderness than EAR. Soysal's [28] applied more force to the meat of the gender groups, (4.74±0.461 kg/cm²) and female (4.94±0.393 kg/cm²), compared to the ones in our study; however he applied less force compared to the cattle with GG genotype. The difference between the Sosyal's [28] TGC sample and ours may be the genotypic differences or the nutrition of the animals. The SF values (9.95–9.97±0.23 kg/cm²) that Preziuso *et al.*[31] obtained from

Limousine cattle and Limousine crossbreeds were two times higher than the values Soysal's [28] (4.85±0.294 kg/cm^2) and the authors (4.483±0.252 kg/cm^2) of this study obtained from TGC beef samples. So, our and Sosyal's [28] TGC samples of meat can be said to have higher tenderness compared to EAR and Limousine beef according to the results of this study. Also, according to Pirenaica and Panda de Montana breeds (bulls 5.1±1.23 and 6.5±1.80 kg/cm^2, respectively) LD muscle SF values, TGC breeds are estimated to have more tender meat than Pirenaica and Parda de Montana bulls although they have tougher meat. When the average SF of TGC samples (4.483±0.252 kg/cm^2) in our study is compared to that of 15 European cattle breeds (5.49±1.25 kg/cm^2) [32] and EAR, TGC seem to have more tender meat. The shearing force applied to the LD muscle of Hanwoo cattle is [27] (-1.32 kg/cm^2) less than the average SF value for TGC.

Previous researchers reported that less force was applied to the LD muscle of the cattle with the *Uo*G-CAST CC genotype than the cattle with CG and GG genotypes and those cattle with CC genotype have more tender meat [5,6,11,22]. SF was applied to LD of Aberdeen Angus, Limousine, Charolais, Simmental and their crossbreeds on the 21st day after death as avarage 4.53±0.12 kg/cm^2 for with the *Uo*G-CAST CC and 4.91±0.15 kg/cm^2 for with GG genotype, and the difference was statistically significant (P<0.05) [5]. The SF average values of *Bos indicus* (Nellore) and *Bos taurus* x *Bos indicus* crossbreeds with the *Uo*G-CAST CC and CG genotypes were 3.47±0.007 kg/cm^2 and 3.63±0.07 kg/cm^2, respectively, and the difference was significant (P<0.05) [11]. Compared to the average SF of TGC (3.943±0.441 kg/cm^2) with the *Uo*G-CAST CC genotype, the Aberdeen Angus, Limousine, Charolais, Simmental and the other crossbreeds with the same genotype that Schenkel *et al.*[5] worked on have less tender meat than TGC; however TGC with the *Uo*G-CAST GG genotype are estimated to yield tougher meat than the other cattle with the same genotype. Also, Nellore breeds with the *Uo*G-CAST CC and CG genotypes [11] are estimated to have more tender meat than TGC with the *Uo*G-CAST GG genotype.

The SF average of Piedmontese, Simmental, Angus, Hereford, Irish, Charolais and their crossbreeds with the CAPN1 316 CC genotype found lower than the other two genotypes (GC and GG) [8,9,13,33]. Brahman breeds have a similar structure also [3,22]. Chung *et al.*[24] found the SF averages with the CAPN1 316 genotypes as CC 4.868±0.44 kg/cm^2, CG 5.180±0.22 kg/cm^2 and GG 5.437±0.27 kg/cm^2 in Hanwoo cattle, which suggests no relationship between the results and the genotypes (P=0.530). TGC breeds also have a similar structure. TGC with the CAPN1 316 GC genotype were found to have more tender meat than TGC with GG genotype but the difference (-0.696) was not statistically significant (P>0.05). We can assume that TGC with the CAPN1 316 GC genotype (3.869±0.721 kg/cm^2) are more likely to have tougher meat than the Piedmontese, Angus and Brahman breeds.

Bos taurus and *Bos taurus* x *Bos indicus* crossbreeds with the CAPN1 4751 CC genotype seem to have more tender meat compared to the other genotypes, and the difference is significant (P<0.005) [4,7,14,16]. Hanwoo cattle with the CAPN1 4751 CC, CT and TT genotypes seem to have the highest (4.946±0.19 kg/cm^2), medium (5.700±0.28 kg/cm^2) and the lowest (5.974±0.72 kg/cm^2) level of meat tenderness, respectively [24]. We can say that TGC with the CAPN1 4751 CT genotype (4.537±1.666 kg/cm^2) are more likely to have less tender meat than the Nellore breeds and *Bos taurus* crossbreeds [4], Brahman calves with the CAPN1 4751 CT [3], and also Piedmontese and Aberdeen Angus with the CAPN1 316 GG [9]. On the other hand, Gill *et al.*[20] claim these the CAPN1 4751 and the *Uo*G-CAST SNPs do not seem to affect meat tenderness in Aberdeen Angus cattle.

Ciobanu *et al.*[34] who worked on pigs and Reardon *et al.*[19] who worked on cattle could not find a correlation between the polymorphisms in CAST gene and WHC. Li *et al.*[10] who worked on the meat of Angus, Charolais, Hereford, Limousine, and Simmental calves in Sweden also could not find a meaningful relationship between the WHC properties; and some of the SNPs genotypes of CAST, CAPN1, LEP, DGAT1 and SCD1 gene. Similarly, we could not find a significant relationship between the genotypic properties of the CAPN1 or CAST SNPs and WHC or CL values of TGC.

In our research was found that the male cattle with CC/CT/GG genotype seemed to have the most tenderness meat with 2.329 kg/cm^2 SF. The previous research carry outed on Hereford, Brahman, Brangus, Red Angus cattle and Charolais X Angus crossbreeds showed that the cattle with GG/TT/GG genotype among the *Uo*G-CAST, the CAPN1 4751 and 316 triple SNPs combination had the toughest meat while the cattle with CC/CC/CC genotype tend to have the most tenderness meat [6,18]. Our findings show that TGC with GG/TT/GG genotypes have the toughest meat and this finding supports the previous studies. TGC carry SNPs that are related to meat tenderness in their CAPN1 and CAST genes, which are polymorphic. Except for the CAPN1 316 CC genotype, all CAST and CAPN1 SNP genotypes, which are associated with meat tenderness, were observed in TGC samples. Based on the SF averages which measure the resistance of the red muscle fibers, we can conclude that TGC yield more tender meat compared to East Anatolian Red, Jersey, Limousine, Pirenaica and Parda de Montana breeds. The presence of genetic variation in specific markers in TGC contributes to the process to yield more tender beef. The purebred TGC with the haplotype which includes heterozygous or double homozygous positive genes in CAST and CAPN1 genes should be kept for breeding. The effects of monadic

genes of TGC which are independent of the potential interactions with the environment on the meat tenderness, which has of economic importance and the productivity, can be designated through crossbreeding. TGC with (C/C/C) haplotype or (CC/CC/CC) genotype carrying the positive genes can be kept for breeding with the use of DNA tests to evaluate the cattle based on the *Uo*G-CAST, the CAPN1 316 and 4751 markers. In order to make TGC breeding a sustainable business, it is necessary to have cattle with more tender beef and to increase the consumption of it. Thus, there is a need to cattle which yield beef of good quality and whose genetic potential is defined. By focusing on more genes and the specific genetic indicators in these genes that are related to the quality of meat, it is possible to improve the precision in selection processes.

Conflicts of Interest

The authors declare no conflict of interest.

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