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The Genetic Characterization of Turkish Grey Cattle with Regard to UoG Cast, CAPN1 316 and CAPN1 4751 Markers

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ABSTRACT

Turkish Grey cattle (TGC) is one of Turkey's native cattle breeds. TGC was a popular race to breeding within Thrace and Western Anatolia, but unfortunately they are under the threat of extinction recently. The application of gene specific molecular markers in genotyping and genetic identification is of essential significance for preserving genetic diversity. The aim of this study was to reveal the genotype profile of indigenous types for TGC breed. In order to characterize TGC population, two polymorphic loci in cattle Calpain gene (CAPN1) and one polymorphic locus in Calpastatin gene CAST were studied. In order to sort the allele variants (G/C), PCR products, the ones in CAST with R*sa*I enzyme, was cut using RFLP method. The allele variants (G/C) of CAPN1 316 and (C/T) of CAPN1 4751 were determined with ARMS-PCR method. This study was conducted to determine three SNPs which were related to meat quality in 130 purebred female and male samples of TGC breed. In TGC samples, C allele frequencies related to UoG-CAST, CAPN1 316 and CAPN1 4751 polymorphisms were found to be 0.6038, 0.1115 and 0.5654, respectively. CC genotype frequencies in the three polymorphic areas in TGC were calculated as 0.365, 0.012 and 0.320, respectively, while the distributions of the marker genotypes, except for CAPN1 4751 were found to be significant ($P<0.05$). The positive alleles of SNPs in Calpain and Calpastatin genes and all of their genotype combinations, were also found to be carried by TGC.

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Authors' Contributions
SK designed the study, analysed the data and wrote the manuscript. SA assisted in designing the study, performed experiments and helped in statistical analysis. HSE and MS helped in performing experiment.

Key words
Grey cattle, CAST, CAPN1, SNP, Genetic marker

INTRODUCTION

The grey cattle (TGC) is believed to be a subpopulation of *Bos taurus primigenius* (Sasimowski, 1987). It is believed that TGC are related to some of the local grey cattle living in Europe. There are many similar local breeds in Europe such as Bulgarian grey cattle in Bulgaria (Hristov *et al*., 2014), Istrian (Croatia), Dalmatian Grey (Croatia), Slovenian podolian (Croatia), Katerin (Greece), Sykia (Greece), Hungarian Grey (Hungary), Cinisara (Italy), MareManna (Italy), Podolicia (Italy), Romanian steppe (Romania), Ukrainian Grey (Ukraine), Istrian (Yugoslavia) and Turkish Grey (Turkey). These are a group of similar breeds according to the European Association Animal Science-Animal Genetic Resources Data Bank resources (Soysal and Kök, 2006). TGC are the only local indigenous race within Marmara Region. Although the number of the cattle raised in Thrace and Western Anatolia was a popular breed, their number is not known for certain and they are under threat of extinction (Soysal *et al*., 2005). A herd of TGC is protected in Bandırma Livestock Research Institute is preserved as an *in-situ* conservation including 12 herds in the 5 provinces by Republic of Turkey Ministry of Food, Agriculture and Livestock.

According to consumer reports, the biggest problem for consumers is the tenderness of the meat (Miller *et al*., 1995). Some of the most important factors that determine the quality of the meat products, thus affecting the opinions of people are the color of the meat and the hygiene conditions, boiling-off loss, the consistency and tenderness of the meat during the phases of purchase, cooking and consumption, respectively (Özdoğan *et al*., 2004). Molecular marker search and development studies that aim to improve the quality of meat directly or indirectly have proven their value recently. Molecular genetics studies are especially useful for improving some of the traits related to the meat quality (Elmacı and Öner, 2007).

Calpain (CAPN1 316 and CAPN1 4751 polymorphisms) and Calpastatin (UoG CAST polymorphism) genes are regarded as the candidate genes for tenderness (Rincón and Medrano, 2006). It was Bishop *et al*. (1993) who first identified Calpastatin as the candidate gene for tenderness. Lonergan *et al*. (1995)

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studied the Calpastatin gene in cattle and called for further research on the effects of variation on Calpastatin activity. In their study, Casas *et al*. (2006) developed CAST and CAPN1 (μ-calpain) genetic markers for the production of more tender (soft) meat to be used with animals whose genetic potentials are defined. The research into the CAST and CAPN1 genetic loci supports the effect of these genes on the tenderness of the meat and it was proved that the genotypic results obtained in SNP studies directly affect the phenotype (Casas *et al*., 2006; Van Eenennaam *et al*., 2007).

Calpastatin gene, depending on the presence of cutting area in the 5th intron of the *Rsa*I restriction endonuclease enzyme detected by restriction fragment length polymorphism (RFLP) method, UoG-CAST polymorphism (AY_008267.1:g.282C>G) was observed to have an effect on the tenderness of the meat. Cattle with CC genotypes tend to have more intermuscular lipoidosis compared to the animals with GG and GC genotypes which increases the tenderness of the meat (Schenkel *et al*., 2006). Many studies in the literature support the fact that the cattle with CC homozygous genotypes are more tender than cattle with other genotypes (Casas *et al*., 2006; Van Eenennaam *et al*., 2007; Schenkel *et al*., 2006; Barendse, 2009; Ribeca *et al*., 2009; Curi *et al*., 2010; Gill *et al*., 2009).

Rincon and Medrano (2006), who studied the Calpain gene, identified CAPN1 316 and CAPN1 4751 genotypes using Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) method. CAPN1 4751 (AF_248054.2:g.6545C>T) polymorphism is in the 17th intron of the CAPN1 gene in cattle. CAPN1 316 (AF 252504:g.5709C>G) polymorphism is the G>C variation in the 9th exon in the CAPN1 gene in cattle. This polymorphism causes amino acid changes in Calpain protein. While C allele codes the alanine amino acid, G allele codes the glycine amino acid. It is possible to increase the tenderness of the meat if the frequency of C/C haplotypes in CAPN1 316/4751 in cattle is increased and the use of these markers can lead to improvements in meat industry (Van Eenennaam *et al*., 2007). The fact that the C allele in both polymorphisms in Calpain gene can be used as the superior allele in the selection studies related to meat tenderness is shown in various cattle breeds (Casas *et al*., 2006; Smith *et al*., 2009; Curi *et al*., 2009; Corva *et al*., 2007; Allais *et al*., 2011; Page *et al*., 2002; Costello *et al*., 2007).

If there is any positive genetic markers highlighting to the meat quality in the TGC, these will be contribute to the sustainability of TGC. Thus in this study, we investigated whether TGC carry positive marker genes for the tenderness of meat.

MATERIAL AND METHODS

Animal samples

This study was carried out on 130 animals of one Turkish beef breed that had no consanguinity indicate. TGC was a popular race to breeding within Thrace and Western Anatolia in Turkey. Animal samples were obtained from different regions during 2013. Tissue samples were taken from 79 pure the TGC that were raised in the villages of Gelibolu and Evreşe Districts of Çanakkale Province and the villages of İpsala and Enez Districts of Edirne Provinces and then, the samples were taken to Keşan Slaughter House to be used as research material in addition to the blood samples of 51 pure TGC within the *ex-situ* protection program from Animal Breeding Research Station of Bandırma.

Samples and DNA isolation

The tissue and blood samples were stored in a deep freezer (-20°C). Laboratory analyses were done in Biotechnology and Genetics Laboratory in the Trakya University. Genomic DNA of the blood and tissue samples taken from 130 purebred TGC were isolated using Fujifilm Quick Gene Mini80 device and commercial kits (QuickGene DNA Tissue-Whole Blood KitS, Fujifilm). Spectrometric $A^{260/280}$ method was used to determine the amount of the DNA that was isolated (Helios Aquamate, Thermo Spectronic).

DNA amplification and genotyping

In order to determine UoG CAST genetic polymorphism, PCR-RFLP method was used based on the work of Schenkel *et al*. (2006). In order to determine CAPN1 4751 and CAPN1 316 genotypes, ARMS-PCR method was used based on the work of Rincon and Medrano (2006). The primers (Sentegen Biotech, Ankara / Turkey) used and fragment sizes that were reproduced (Table I) by Bioneer My Genie 96 Thermal Block PCR device (Bioneer Corporation, South Korea). All PCR reactions were performed EmeraldAmp GT PCR master mix (Takara, Japan). Amplification of DNA was run in the 3% horizontal gel electrophoresis by "Thermo Scientific electrophoresis and power supply" and then was used for the genotyping by "DNR BioImaging Systems Minibis Pro. Jerusalem, Israel".

Statistical analyses

In our study where the SNPs (single nucleotide polymorphisms) were identified in the one and two loci of the Calpastatin and Calpain genes, respectively, the frequencies of the alleles and genotypes were calculated

Table I.- The PCR method used, primer sequences and amplification products.

Marker	Sequences of the primers (5' - 3')	bp***
UoG CAST*	[1]Fop:CTCGACTGCGTACCAATTCCGAAGTAAAGCCAAAGGAACA	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	

*, Schenkel *et al.* (2006) used RFLP; **, Rinco´n and Medrano (2006) used ARMS method; ***, PCR product size (bp); [1]Fop, Forward outer primer; [2]Rop, Reverse outer primer; [3]Fip, Forward inner primer; [4]Rip, Reverse inner primer.

with the use of Popgene32 (version 1.31) based on Hardy-Weinberg equilibrium (Wright, 1969).

RESULTS AND DISCUSSION

Genotypic distribution and allele frequencies in UoG-CAST locus

Both of the alleles (C and G) of the *U*oG-CAST polymorphism and three genotypic structures (CC, CG, GG) were found in the CAST locus in TGC (Fig. 1).

General mean frequency (0.3962) of the G allele in TGC population was studied that was observed to be low. The allele frequencies of the UoG-CAST polymorphism were calculated according to Hardy-Weinberg equilibrium (Table II). The mean frequency of the C allele was calculated as 0.6038 in our study. In earlier studies they conducted on UoG-CAST SNP in cattle populations of *Bos taurus* cross-breeding and *Bos taurus* origin, Schenkel *et al*. (2006), Savaşçı and Atasoy (2016), Quaas *et al*. (2006), Van Eenennaam *et al*. (2007), Gill *et al*. (2009), Curi *et al*. (2010) and Reardon *et al*. (2010) found the mean frequency of the C allele which has a positive effect on tenderness of the meat as 0.629, 0.67, 0.72, 0.72, 0.64, 0.693 and 0.75, respectively. The frequency of the C allele in UoG-CAST locus in TGC was found below the value (0.72) reported by Savaşçı and Atasoy (2016), whereas it was higher than the one (0.507) reported by Kök *et al*. (2013).

The average genotypic frequencies (0.365, 0.478 and 0.157 for CC, CG and GG, respectively) were observed to be in compliance with Hardy-Weinberg equilibrium (Table II) and the gene frequency difference were found significant ($P<0.01$).

The genotypic frequencies for UoG-CAST

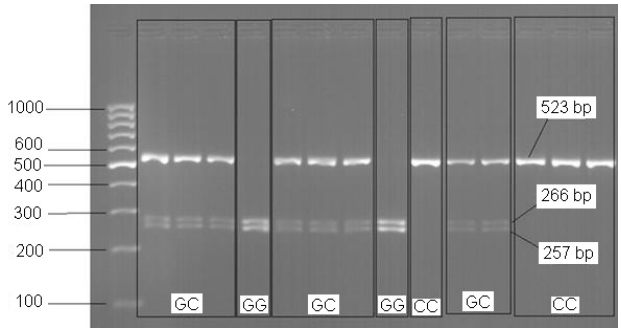

Fig. 1. The view of the genotypes in agarose gel electrophoresis of *U*oG-CAST polymorphism with PCR-RFLP method.

Table II.- The allele and genotypes frequencies of Calpastatin (UoG-CAST) and Calpain (CAPN1 4751, CAPN1 316) genes in the Turkish grey cattle.

Allele		Genotype		
		U*oG-CAST		
C	G	CC	CG	GG
0,6038	0,3962	0,365	0,478	0,157
		CAPN1 4751*		
C	T	CC	CT	TT
0,5654	0,4346	0,320	0,491	0,189
		CAPN1 316*		
C	G	CC	CG	GG
0,1115	0,8885	0,012	0,198	0,789

*n, Number of TGC were 130 head.

polymorphism in Aberdeen Angus cattle were reported by Gill *et al*. (2009) as 0.41, 0.47 and 0.12 for CC, CG and GG, respectively. Schenkel *et al*. (2006) reported the CC, CG and GG genotypic frequencies for *Bos taurus* cattle sample as 0.430, 0.398 and 0.172, respectively. On the other hand, Curi *et al*. (2010) indicated the frequencies for CC, CG and GG genotypes in purebred Nellore cattle as 0.377, 0.491 and 0.132, respectively. Kök *et al*. (2013) found the frequencies of the same genotypes in purebred TGC as 0.257, 0.499 and 0.243, respectively while they calculated the frequencies as 0.388, 0.470 and 0.142 for all the samples including purebred and crossbred cattle. In another research done by Savaşçı and Atasoy (2016), the same genotypic frequency distribution in local breeds were found as 0.45, 0.47 and 0.08, respectively while the results were found as 0.50, 0.44 and 0.06 for TGC.

When the average of the UoG-CAST polymorphism CC genotype (0.365) frequencies is compared to the findings of the other researchers, it is higher than the average reported by Kök *et al*. (2013) while it falls below the average calculated by Savaşçı and Atasoy (2016). GG genotype frequency (0.157) was found close to the frequency results of the other researchers except for Savaşçı and Atasoy (2016).

The mean frequency of the positive C allele to meat tenderness in TGC was observed to be high. Reproduction of CAST CC genotype in TGC population using selection to increase the number of the cattle with more tender meat seems logical as it is supported by the findings of many other researchers (Curi *et al*., 2010; Gill *et al*., 2009; Van Eenennaam *et al*., 2007; Schenkel *et al*., 2006; Casas *et al*., 2006) when the positive correlation between CAST CC genotype and the phenotypic results obtained via Warner-Bratzler Shear Force (WBSF) device.

Genotypic distribution and allele frequencies in calpain CAPN1 4751 locus

Both of the genetic variants (C and T) were observed in the CAPN1 4751 polymorphism in the Calpain genes of TGC sample. Based on these variants, CC, CT and TT genotypes were identified in CAPN1 4751 locus in TGC (Fig. 2).

The C allele (0.5654) in TGC sample was found to have higher frequency compared to the T allele (0.4346). CC, CT and TT genotype frequencies in CAPN1 4751 locus were found 0.320, 0.491 and 0.189, respectively (Table II). The distribution difference of the genotype frequencies in TGC was not found statistically significant (P>0.05). White *et al*. (2005) found a value between 0.575 and 0.639 for the CAPN1 4751 C allele frequency, which has a positive effect on meat tenderness, in *B. taurus* cattle. Smith *et al*. (2009) found 0.05 for Brahman bullock while Van Eenennaam *et al*. (2007) obtained a value ranged between 0.06 and 0.64 for Charolais x Angus, Brangus, Red Angus, Brahman, Hereford cattle populations. On the other hand, Curi *et al*. (2009) who studied Nellore, Angus x Nellore, Rubia Gallega x Nellore, Canchim, Brangus cross-breedings and Braunvieh cattle determined the average of the population as 0.205 (0.105 – 0.421).

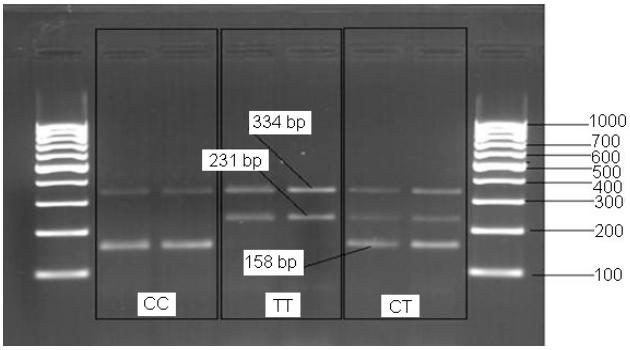

Fig. 2. The view of the genotypes in agarose gel electrophoresis of CAPN1 4751 polymorphism with ARMS-PCR method.

CAPN1 4751 CC, CT and TT genotype frequencies were found 0.41, 0.48 and 0.11, respectively for Aberdeen Angus cattle by Gill *et al*. (2009) while Frylick *et al*. (2009) who worked on Brahman cross-breeding and Simmental cross-breeding obtained the values of 0.53, 0.263, 0.684 and 0.05, 0.35, 0.60, respectively. Curi *et al*. (2010) who investigated the same genotypes found 0.00, 0.21 and 0.79 for the Nellore cattle and 0.073, 0.561 and 0.366 for the Canchim cattle they worked on. In respect of frequency of the positive CC genotype identified in CAPN1 4751 locus in TGC population, it was lower than the values found by Gill *et al*. (2009) in Aberdeen Angus studied and by Frylinck *et al*. (2009) in Brahman cross-breeding while it was higher than the genotypic frequencies of the sample populations of Simmental cross-breeding and the Nellore and Canchim cattle studied by Curi *et al*. (2010).

The average CAPN1 4751 C allele frequency for our sample TGC population is 0.5654 and it is higher than the results obtained by all the other researchers except White *et al*. (2005). This percentage supports the idea that the number of the cattle in the population carrying CAPN1 4751 C allele, which is positively related to meat tenderness, constitutes the majority. Reproduction of CAPN1 4751 CC and CT genotypes in TGC population using selection to increase the number of the cattle with more tender meat seems logical as it is supported by the findings of many other researchers (Van Eenennaam *et*

al., 2007; Allais *et al*., 2011; Page *et al*., 2002) when the positive correlation between CAPN1 4751 CC genotype and the phenotypic results obtained via WBSF. This will also increase the meat consumption in the areas where TGC are raised.

Genotypic distribution and allele frequencies in calpain CAPN1 316 locus

Both of the genetic variants (C and G) were observed in the CAPN1 316 polymorphism in the Calpain genes of TGC sample. Based on these variants, CC, CG and GG genotypes were identified in CAPN1 316 locus in TGC (Fig. 3).

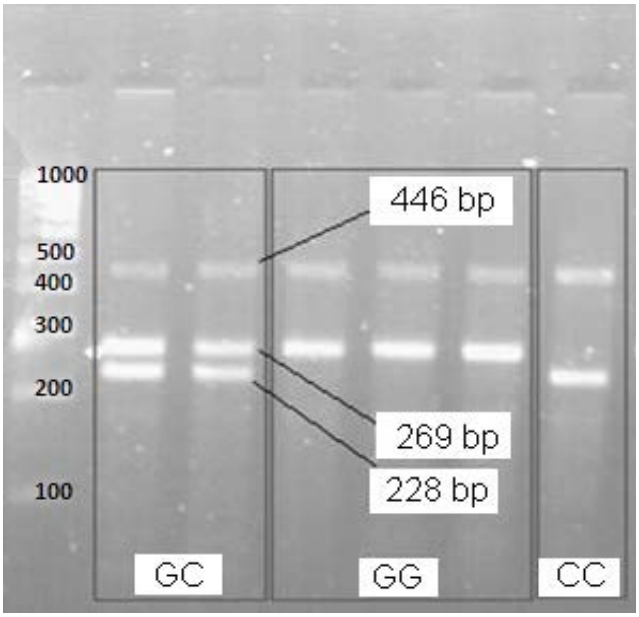

Fig. 3. The view of the genotypes in agarose gel electrophoresis of CAPN1 316 polymorphism with ARMS-PCR method.

The CAPN1 316 C allele frequency (0.1115) in the total sample of TGC was found to have much lower frequency compared to the G allele (0.8885). CC, CG and GG genotype frequencies in CAPN1 316 locus were found 0.012, 0.198 and 0.789, respectively (Table II). Among the 130 samples of TGC, only 2 cattle were identified as CC genotype. The distribution difference of the genotype frequencies in TGC was found statistically significant ($P<0.001$).

Curi *et al*. (2010), Page *et al*. (2004), Smith *et al*. (2009), Gill *et al*. (2009), Allais *et al*. (2011), Corva *et al*. (2007) and Morris *et al*. (2006) identified the C allele which is positively correlated to meat tenderness in CAPN1 316 SNP in *B. taurus* and *B. taurus* x *B. indicus* populations as 0.06, 0.17-0.20, 0.031, 0.22, 0.04-0.27, 0.29-0.46 and 0.23-0.68, respectively. CAPN1 316 C allele frequency in Taurine and Zebu cattle was reported to be smaller than the G allele frequency while it has always been observed to be higher in *B. taurus* cattle and have a positive correlation with meat tenderness (Van Eenennaam *et al*., 2007; Curi *et al*., 2010; Gill *et al*., 2009; White *et al*., 2005; Page *et al*., 2002, 2004; Morris *et al*., 2006; Casas *et al*., 2005). CAPN1 316 C allele frequency of TGC sample in our research falls below the one found by Gill *et al*. (2009) for Aberdeen Angus cattle (0.22) while it seems to be higher than Brahman cross-breeding (0.025) and Simmental cross-breeding (0.05) that were investigated by Frylinck *et al*. (2009) and *B. taurus* x *B. indicus* cross-breeding (0.06) that were studied by Curi *et al*. (2010). TGC sample used in our study was observed to be similar to *B. indicus* with regard to CAPN1 316 C allele (0.1115) frequency.

CAPN1 316 CC, CG and GG genotypes frequencies were investigated by Gill *et al*. (2009) who found 0.05, 0.35 and 0.61 in Aberdeen Angus cattle, by Frylinck *et al*. (2009) who found in 0.00, 0.05, 0.95 and 0.00, 0.10, 0.90, respectively in Brahman and Simmental cross-breeding and by Curi *et al*. (2010) who worked with B. *taurus* x B. *indicus* cross-breeding and found 0.00, 0.12 and 0.88. The CC genotype frequency (0.012) is positively correlated to meat tenderness in CAPN1 316 locus in TGC population is smaller than the frequency of the Aberdeen Angus cattle with which Gill *et al*. (2009) worked while it is higher than the genotypic frequency of the Brahman cross-breeding (0.025) and Simmental cross-breeding (0.05) that were investigated by Frylinck *et al*. (2009) and the *B. taurus* x *B. indicus* cross-breeding (0.06) that were studied by Curi *et al*. (2010).

CONCLUSION

All of the allelic variants (*U*oG-CAST C/G and CAPN1 316 C/G or CAPN1 4751 C/T) were determined and some of the genotypic combinations of *U*oG-CAST, CAPN1 316 and CAPN1 4751 gene polymorphism were observed in TGC. The positive *U*oG-CAST CC, CAPN1 316 CC and CAPN1 4751 CC genotype frequencies in those three polymorphic areas in TGC were calculated as 0.365, 0.012 and 0.320, respectively. The haplotype C/C/C frequency of the three markers for TGC population that carries the positive genes for meat tenderness was calculated as 0.0381. However, there were no cattle found with homozygous CC/CC/CC genotype in none of the three polymorphic loci of TGC sample. In order to raise cattle with more tender meat, the positive haplotype frequency needs to be increased in herds. It is recommended to make use of selection processes to raise cattle with homozygous

CC/CC/CC genotypes in all the three loci in TGC that are to be kept for breeding and meat.

RECOMMENDATIONS

As TGC are assumed to carry positive allele genes and have more tender meat than the other cattle, an important contribution can be made to the sustainability of TGC population by increasing their number which brings meat production characteristics of TGC to the front. SNPs independent of the potential interactions with environmental factors can be utilized to improve the characteristics that are of economic importance, which makes the comparison of crossbred and inbred cattle possible giving the opportunity to choose the parents-to-be to raise cattle with more tender meat. As in countries with most developed breeding by livestock, homozygous purebred mother and father lines can be formed to improve the quality of the beef in Turkey, as well. It will also contribute to the identification and improvement of the meat tenderness with MAS. Furthermore, the genotypes identified in this study should be supported with phenotypic testing studies on *longissimus dorsi* muscle (entrecote) in TGC with WBSF device as a subject of future research.

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Conflict of interest statement

We declare that we have no conflict of interest.

REFERENCES

Allais, S., Journaux, L., Leveziel, H., Payet-Dprat, N., Raynaund, P., Hocquette, J.F., Lepetit, J., Rousset, S., Denoyelle, C., Bernard-Capel, C. and Renand, G., 2011. Effects of polymorphisms in the calpastatin and μ-calpain genes on meat tenderness in 3 French beef breeds. *J. Anim. Sci.,* **89**: 1-11. http://dx.doi.org/10.2527/jas.2010-3063

Barendse, W. J., 2009. *DNA markers for meat tenderness*. United States Patent 625698, Available at: http://www.freepatentsonline.com/7625698.html (accessed 17 June 2016).

Bishop, M.D., Koohmaraie, M., Killefer, J. and Kappes, S., 1993. Rapid communication: Restriction fragment length polymorphisms in the bovine calpastatin gene. *J. Anim. Sci.,* **71**: 2277.

Casas, E., White, S.N., Riley, D.G., Smith, T.P.L., Brenneman, R.A., Olson, T.A., Johnson, D.D., Coleman, S.W., Bennett, G.L. and Chase Jr, C.C., 2005. Assessment of single nucleotide polymorphisms in genes residing on chromosomes 14 and 29 for association with carcass composition traits in *Bos indicus* cattle. *J. Anim. Sci.,* **83**: 13–19. http://dx.doi.org/10.2527/2005.83113x

Casas, E., White, S.N., Wheeler, T.L., Shackelford, S.D., Koohmaraie, M, Riley, D.G., Chase, J.C.C., Johnson, D.D. and Smith, T.P.L., 2006. Effects of calpastatin and micro-calpain markers in beef cattle on tenderness traits. *J. Anim. Sci.,* **84**: 520−525. http://dx.doi.org/10.2527/2006.843520x

Corva, P., Soria, L., Schor, A., Villarreal, E., Cenci, M.P., Motter, M., Mezzadra, C., Melucci, L., Miquel, C., Paván, E., Depetris, D., Santini, F. and Naón, J.G., 2007. Association of CAPN1 and CAST gene polymorphisms with meat tenderness in *Bos taurus* beef cattle from Argentina. *Genet. Mol. Biol.,* **30**: 1064−1069. http://dx.doi.org/10.1590/S1415-47572007000600006

Costello, S., O'Doherty, E., Troy, D.J., Ernst, C.W., Kim, K.S., Stapleton, P., Sweeney, T. and Mullen, A.M., 2007. Association of polymorphisms in the calpain I, calpain II and growth hormone genes with tenderness in bovine *M. longissimus dorsi*. *Meat Sci*., **75**: 551-557. http://dx.doi.org/10.1016/j.meatsci.2006.06.021

Curi, R.A., Chardulo, L.A.L., Giusti, J., Silveira, A.C., Martins, C.L. and Oliveira, H.N., 2010. Assessment of GH1, CAPN1 and CAST polymorphisms as markers of carcass and meat traits in *Bos indicus* and *Bos taurus–Bos indicus* cross beef cattle. *Meat Sci*., **86**: 915–920. http://dx.doi.org/10.1016/j.meatsci.2010.07.016

Curi, R.A., Chardulo, L.A.L., Mason, M.C., Arrigoni, M.D.B., Silveira, A.C. and Oliveira, H.N., 2009. Effect of single nucleotide polymorphisms of CAPN1 and CAST genes on meat traits in Nellore beef cattle (*Bos indicus*) and in their crosses with Bos taurus. *Anim. Genet*., **40**: 456−462. http://dx.doi.org/10.1111/j.1365-2052.2009.01859.x

Elmacı, C. and Öner, Y., 2007. Molecular genetic approaches in beef cattle. *Anim. Prod.,* **48**: 45-48.

Frylinck, L., vanWyka, G.L., Smith, T.P.L., Strydom, P.E., van Marle-Köster, E., Webb, E.C., Koohmaraie, M. and Smith, M.F., 2009. Evaluation of biochemical parameters and genetic markers for association with meat tenderness in South African feedlot cattle. *Meat Sci*., **83**: 657–665. http://dx.doi.org/10.1016/j.meatsci.2009.07.016

Gill, J.L., Bishop, S.C., McCorquodale, C., Williams, J.L. and Wiener, P., 2009. Association of selected SNP with carcass and taste panel assessed meat quality traits in a commercial population of Aberdeen Angus-sired beef cattle. *Genet. Sel. Evol*., **41**: 1-12. http://dx.doi.org/10.1186/1297-9686-41-36

Hristov, P.I., Teofanova, D.R., Neov, B.S., Zagorchev, L.I. and Radoslavov, G.A., 2014. Population structure of two native Bulgarian cattle breeds with regard to *CSN3* and *CSN1S1* gene polymorphism. *Bulg. J. Vet. Med.*, **17**: 18−24.

Kök. S., Atalay, A., Savaşçı, M. and Eken, H.S., 2013. Characterization of calpastatin gene in purebred and crossbred Turkish grey steppe cattle. *Kafkas Univ. Vet. Fak. Derg*., **19**: 203-206. http://dx.doi.org/10.9775/kvfd.2012.7470

Lonergan, S.M., Ernst, C.W., Bıshop, M.D., Calkins, C.R. and Koohmaraie, M., 1995. Relationship of restriction fragment length polymorphisms (RFLP) at the bovine calpastatin locus to calpastatin activity and meat tenderness. *J. Anim. Sci.,* **73**: 3608-3612. http://dx.doi.org/10.2527/1995.73123608x

Miller, M.F., Huffman, K.L., Gilbert, S.Y., Hamman, L.L. and Ramsey, C.B., 1995. Retail consumer acceptance of beef tenderized with calcium chloride. *J. Anim. Sci.,* **73**: 2308–2314. http://dx.doi.org/10.2527/1995.7382308x

Morris, C. A., Cullen, N. G., Hickey, S. M., Dobbie, P. M., Veenvliet, B. A., Manley, T. R., Pitchford, W. S., Kruk, Z. A., Bottema, C. D. K. and Wilson, T., 2006. Genotypic effects of calpain 1 and calpastatin on the tenderness of cooked *M. longissimus dorsi* steaks from Jersey×Limousin, Angus and Hereford-cross cattle. *Anim Genet*., **37**: 411-414. http://dx.doi.org/10.1111/j.1365-2052.2006.01483.x

Özdoğan, M., Önenç, A., Önenç, S.S. and Köknaroğlu, H., 2004. *The effect of feeding on beef quality*. 4th National Animal Science Congress, Isparta, Turkey, pp. 517-523.

Page, B.T., Casas, E., Heaton, M.P., Cullen, N.G., Hyndman, D.L., Morrıs, C.A., Crawford, A.M., Wheeler, T.L., Koohmaraie, M., Keele, J.W. and Smith, T.P.L., 2002. Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. *J. Anim. Sci.,* **80**: 3077-3085. http://dx.doi.org/10.2527/2002.80123077x

Page, B.T., Casas, E., Quaas, R.L., Thallman, R.M., Wheeler, T.W., Shackelford, S.D., Koohmaraie, M., White, S.N., Bennett, G.L., Keele, J.W., Dikeman, M.E. and Smith, T.P.L., 2004. Association of markers in the bovine CAPN1 gene with meat tenderness in large crossbred populations that sample influential industry sires. *J. Anim. Sci.,* **82**: 3474–3481. http://dx.doi.org/10.2527/2004.82123474x

Quaas, R.L., Li, J., Thallman, R.M., Van Eenennaam, A.L., Fernando, R.L. and Gill, C., 2006. *Validation of commercial DNA tests for quantitative beef traits*. 8th World Congress on Genetics Applied to Livestock Production, 13-18th August, Belo Horizonte, MG, Brasil, pp. 18.

Reardon, W., Mullen, A.M., Sweeney, T. and Hamil, R.M., 2010. Association of polymorphisms in candidate genes with colour, water-holding capacity and composition traits in bovine M. longissimus and M. Semimembranosus. *Meat Sci*., **86**: 270–275. http://dx.doi.org/10.1016/j.meatsci.2010.04.013

Ribeca, C., Bıttante, G., Albera, A., Bonfattı, V., Maretto, F. and Gallo, L., 2009. Investigation on variability of candidate genes for meat quality traits in piemontese. *Ital. J. Anim. Sci*., **8**: 132-134.

Rincón, G. and Medrano, J. F., 2006. Assays for genotyping single nucleotide polymorphisms in the bovine CAPN1 gene. *Anim. Genet*., **37**: 94–295. http://dx.doi.org/10.1111/j.1365-2052.2006.01430.x

Sasimowski, E., 1987. *Animal breeding and production*. Science publishers, B.V. 0-444-99504-8, Elsevier, NL.

Savaşçı, M. and Atasoy F., 2016. The investigation of calpastatin and thyroglobulin gene polymorphisms in some native cattle breeds. *Ankara Üniv. Vet. Fak. Derg.*, **63**: 53-59. http://dx.doi.org/10.1501/Vetfak_0000002709

Schenkel, F.S., Miller, S.P., Jiang, Z., Mandell, I.B., Ye, X., Li, H. and Wilton J.W., 2006. Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *J. Anim. Sci.,* **84**: 291−299. http://dx.doi.org/10.2527/2006.842291x

Smith, T., Thomas, M.G., Bidner, T.D., Paschal, J.C. and Franke, D.E., 2009. Single nucleotide polymorphisms in Brahman steers and their association with carcass and tenderness traits. *Genet. Mol. Res*., **8**: 39-46. http://dx.doi.org/10.4238/vol8-1gmr537

Soysal, İ., Özder, M., Kök, S. and Soysal, D., 2005. *Place in the ecosystem of Turkish grey cattle*. 5th Industrialization and Environment Symposium in Thrace. TMMO Publishing, Edirne, Turkey, pp. 429-440.

Soysal, M.İ. and Kök, S., 2006. *The last survivors of*

grey cattle's whose resisting not to be extincted. A case study of characteristics and sustainability of traditional system of native grey cattle breed. 2nd Seminar of the scientific-Professional Network on Mediterranean Livestock Farming. Mediterranean Livestock Production: Uncertainties and Opportunities, CIHEAM Publishing, Zaragoza, Spain, pp. 55-63.

Van Eenennaam, A.L., Li, J., Thallman, R.M., Quaas, R.L., Dikeman, M.E., Gill, C.A., Franke, D.E. and Thomas, M.G., 2007. Validation of commercial DNA tests for quantitative beef quality traits. *J. Anim. Sci.,* **85**: 891−900. http://dx.doi.org/10.2527/jas.2006-512

White, S.N., Casas, E., Wheeler, T.L., Shackelford, S.D., Koohmaraie, M., Riley, D.G., Chase Jr, C.C., Johnson, D.D., Keele, J.W. and Smith, T.P.L., 2005. A new SNP in CAPN1 is associated with tenderness in cattle of *Bos indicus*, *Bos taurus*, and crossbred descent. *J. Anim. Sci.,* **83**: 2001-2008. http://dx.doi.org/10.2527/2005.8392001x

Wright, S., 1969. *The theory of gene frequencies*. *III: Evolution and the genetics of populations*, Volume 2, University of Chicago Press, Chicago, USA.