MJAVL
Manas Journal of Agriculture Veterinary and Life Sciences
ISSN 1694-7932 | e-ISSN 1694-7932
Volume 11 (Issue 1) (2021) Pages 89-93

Investigation of the CAST Gene Polymorphism in Karayaka Sheep

Koray KIRIKÇI[1a]* Mehmet Akif ÇAM[2b] Levent MERCAN[3c]

[1]Department of Animal Science Faculty of Agriculture Kırşehir Ahi Evran University Kırşehir, TURKEY
[2]Department of Animal Science Faculty of Agriculture Ondokuz Mayıs University Samsun, TURKEY
[3]Department of Agricultural Biotechnology Faculty of Agriculture Ondokuz Mayıs University Samsun, TURKEY

[a]*https://orcid.org/ 0000-0001-8087-141X* [b]*https://orcid.org/ 0000-0003-3407-3913*
[c]*https://orcid.org/ 0000-0002-6790-1458*

*Corresponding author: koray.kirikci@ahievran.edu.tr

ABSTRACT

The study aimed to investigate the CAST gene's genetic polymorphism, known as a candidate gene for meat quality and quantities, using the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method in Karayaka sheep. The blood samples were collected from 105 animals belong to four subpopulations (Samsun, Ordu, Giresun, and Tokat) in Turkey's Black Sea region provinces. The genomic DNA was isolated by using an extraction kit. A fragment of 622 bp on CAST gene was amplified by PCR and then genotypes of the CAST gene for all individuals were determined with the restriction endonuclease *MspI*. At the end of the work, MM and MN's genotypic frequencies were found to be 84 and 16% for the Karayaka breed. While the MN genotype was not observed only in the Ordu subpopulation, the NN genotype was not observed in any subpopulations studied. As a result, the obtained findings provided information about the CAST gene polymorphism for Karayaka breed at the population level. This result could be considered for genomic selection works to improve the meat quality traits of Karayaka in the future.

ARTICLE INFO

Research article
Received:
21.11.2020
Accepted:
21.04.2021

Keywords:

Karayaka sheep, calpastatin gene, genetic variability, PCR-RFLP, *MspI*

INTRODUCTION

Sheep meat is an essential mainstay for farmers in Turkey. The sheep population raised in the Black Sea region is estimated to be 4-5 percent of Turkey's total sheep population 37 276 050 in 2019 (TUIK, 2019). Karayaka breed is raised mainly along the Black Sea region of Turkey, especially in Samsun, Ordu, Giresun, and Tokat provinces. Farmers highly appreciated this breed due to its high meat quality (Olfaz et al. 2005). Because of its desired meat, Karayaka breed attracted the attention of researchers, and the studies on the determination of its meat quality and carcass composition have been progressively continued (Oğan, 2000; Cam et al. 2007; Olfaz et al. 2005; Aksoy et al. 2019).

Meat quality is one of the most important traits in farm animals, is affected by many factors such as genetic and environment (Gao et al. 2007). The meat quality traits' genetic improvement is difficult using traditional selection methods. Because the heritability of meat quality is low and measuring is difficult and only possible after slaughter (Gao et al. 2007). Determining the genes affected on meat quality and applying those to animal breeding is quite important for livestock breeding.

The development of molecular techniques has led to increasing the identification of genes that affects meat quality (Gao et al. 2007) and economically important traits. Those improvements have led to increasing the studies of identifying the genes related to meat quality (Zhang et al. 2014; Sun et al. 2015; Ardicli et al. 2017; Grochowska et al. 2019).

Calpastatin is one of the genes which affects meat quality. Thus, it is among the most studied genes in livestock. A variety of studies have investigated the polymorphisms of CAST gene and its associations with meat quality traits in livestock (Khan et al. 2012; Yilmaz et al. 2014b; Kumar et al. 2016; Ardicli et al. 2017).

Calpastatin was first determined in the Dorset Down sheep breed by Palmer et al. (1998). This gene is located on the sheep fifth chromosome and has two variants named M and N which could be detected by PCR-RFLP technique (Palmer et al. 1998). Calpastatin, an endogenous inhibitor of calpain, has a vital role in meat tenderness after slaughter.

To Cite: Kırıkçı K, Çam MA, Mercan L 2021. Investigation of the CAST Gene Polymorphism in Karayaka Sheep, MJAVL Sciences. 11 (1) 89-93

Associations of Calpastatin gene polymorphisms with the traits of carcass, meat quality and its effects on growth have been reported by researchers (Nikmard et al. 2012; Suleman et al. 2012; Gregula-Kania et al. 2019). Because of these effects, it is known as a major gene.

Some researchers reported polymorphisms of the CAST/*MspI* gene in some Turkish sheep breeds (Yilmaz et al. 2014a; Balcıoglu et al. 2014; Dincel et al. 2015; Ardicli et al. 2017). However, no study has included the polymorphism of the CAST gene at the population level in Karayaka sheep. Therefore, the present study aims to determine the frequencies of genotype and allele of CAST gene and evaluate those at the population level.

MATERIAL AND METHODS

Sampling and DNA isolation

A total of 105 blood samples, previously collected from sheep raised in four populations; Samsun (45), Ordu (16), Giresun (21), and Tokat (23), were used in the present study. DNA isolations from blood was performed using an IDPURE Spin Column Genomic DNA MiniPrep Kit (Empire Genomics, Buffalo, NY) by following the the manufacturer's instructions.

Polymerase chain reaction (PCR)

The PCR reaction was performed in a final volume of 20 µl containing 4 ul of 5x HOT FIREpol® Blend Master Mix, 1 µl of each primer (10 pmol / ul), 2 µl of total DNA (30–50 ng) and finally added ultrapure water until a total volume of 20 µl. The sheep CAST gene was amplified with the primer pairs; 5'-TGGGGCCCAATGACGCCATCGATG-3' and reverse 5'GTGGAGCAGCACTTCTGATCACC-3' using SimpliAmp thermal cycler (Applied Biosystem) (Palmer et al. 1999). To activate polymerase at the beginning of the PCR cycle was performed an incubation step at 95 ^{o}C for 12 min, followed by 1 cycle of 95 ^{o}C 1 min, annealing at 64 ^{o}C for 1 min and an extraction step at 72 ^{o}C for 1 min followed by 35 cycles and 10 min at 72 ^{o}C as a final extraction.

Genotyping of CAST gene

A fragment of 622 bp of the CAST gene was digested by the restriction endonuclease enzyme *MspI* (EUR_X®). The PCR products were incubated for 1 h at 37 ^{o}C in a final volume of 50 µl, involving 10 µl of PCR product, 5 µl of 10x ONE Buffer, 0.5 µl of BSA [100x], 0.3 µl of *MspI* enzyme and finally added ultrapure water until final volume and after digestion heated for inactivation for 20 min at 65 ^{o}C. The digested products were subjected to 3 % agarose gel electrophorese stained with EtBr (500 µ/ml in H_2O) in Figure 1.

Data analysis

Allele and genotype frequencies, expected and observed heterozygosity values, and Hardy-Weinberg test for the studied populations were calculated using PopGene32 software program version 1.32 (Yeh et al. 2000).

RESULTS

In the present study, the genotype and allele frequencies, observed and expected heterozygosity values for both breed and the studied populations were given in Table 1. Based on the PCR-RFLP results for CAST/*MspI*, two genotypes were observed as shown in Figure 1. MM genotypes showed two bands; 287 bp and 336 bp, while MN genotypes showed three bands; 287 bp, 336 bp and 622 bp as seen in Figure 1.

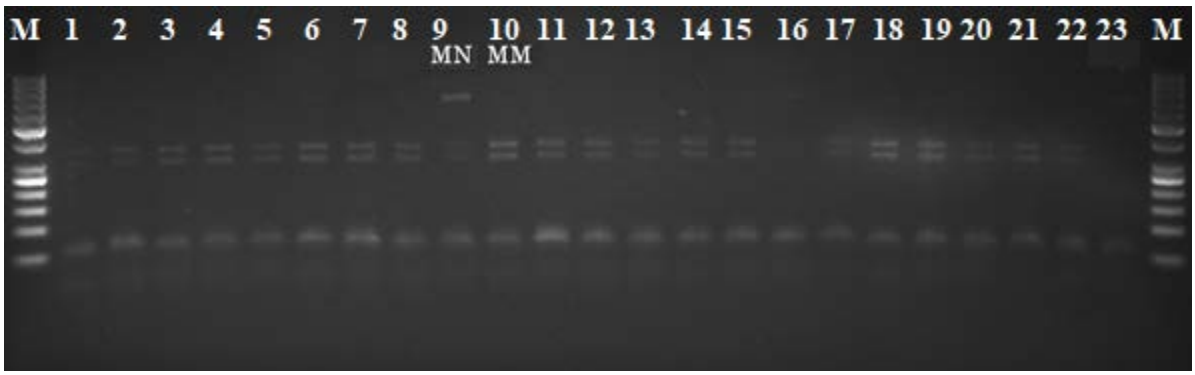

Figure1. *Agarose gel images of the CAST/ MspI genotypes. M: 50 bp DNA ladder, Line 1-8, 10-15,17-22; MM genotypes, Line 9; MN genotypes*

The frequencies of allele and genotype were estimated for the studied populations and summarized in Table 1. The frequencies of MM and MN genotypes for Karayaka breed were calculated as 0.84 and 0.16.

For populations, the MM and MN genotype frequencies were estimated to be 0.78 and 0.22 for Samsun, 0.81 and 0.19 for Giresun, 0.87 and 0.13 for Tokat, respectively. Nevertheless, MN genotype was not observed in the Ordu population and MM frequency was 1.0. The allele frequencies for Karayaka breed were 0.92 and 0.08 for M and N, respectively. Generally, M allele frequency was the highest, ranged from 0.89 in Samsun to 1 in Ordu, with a mean frequency of 0.92. Observed (Ho) and expected (He) heterozygosity values were 0.162 and 0.150. The highest heterozygosity value was observed in the Samsun population as seen in Table 1.

Table 1. *Allele and genotype frequencies, heterozygosity values for the studied populations.*

Populations	N	Allele Frequency (%)		Genotype frequency (%)		Heterozygosity			P
		M	*N*	*MM*	*MN*	*Ho*	*He*	X^2	
Samsun	45	0.89	0.11	0.78	0.22	0.222	0.200	0.627	NS
Ordu	16	1.0	0.0	1	0	0.000	0.000	NE	NE
Giresun	21	0.90	0.10	0.81	0.19	0.191	0.177	0.170	NS
Tokat	23	0.93	0.07	0.87	0.13	0.130	0.125	0.073	NS
Total	**105**	**0.92**	**0.08**	**0.84**	**0.16**	**0.162**	**0.150**	**0.763**	**NS**

Ne= not estimated, Ho = observed heterozygosity, He = expected heterozygosity X^2 = Chi-square, P= probability

DISCUSSION

The CAST gene's genetic polymorphisms in some Turkish sheep breeds have shown and discussed by some researchers (Yilmaz et al. 2014a; Yilmaz et al. 2014b; Balcıoglu et al. 2014; Dincel et al. 2015; Avanus 2015). In the study, MM genotype was the most common genotype (84%) while MN (16%) was the lowest. The MN genotype was not found in the Ordu population, and also, genotype NN was not observed in the Karayaka breed. This may be the result of homozygosity in the population concerning the M allele. This result agreed with those reported by Gabor et al. (2009) and Yilmaz et al. (2014a).

The frequencies of MM and MN genotypes in the current study were similar to the Russian sheep breed (MM: 0.855 and MN: 0.145) reported by Kulikova et al. (2018). The M and N allele frequencies were also found in similar to those (M: 0.928 and N: 0.072) in the same study. The M allele frequency in the present study was higher than being in Prydniprovska sheep (83%), Karakul sheep (79%), Lori sheep (63.8 %), Kajli sheep (81%), Zel sheep (75%) and Polish Merino sheep (76%) (Eftekhari Shahroudi et al. 2006; Khederzadeh 2011; Szkudlarek-Kowalczyk et al. 2011; Nanekarani et al. 2011; Khan et al. 2012; Suleman et al. 2012). The N allele frequency was lower than those reported by some researchers (Nanekarani et al. 2011; Szkudlarek-Kowalczy et al. 2011; Gharahveysi et al. 2012; Suleman et al. 2012; Pomitun et al. 2019). In the current study, the existence of the N allele was no observed only in the Ordu subpopulation.

In the study, the frequency of the MM genotype was found to be higher than those in some Turkish sheep breeds, including Kivircik (40.0), Karakul (46.7), Akkaraman (57.2), İvesi (0.50), Güney Karaman (0.52), Sakız (9.2) and Karacabey Merino (66.9) (Yilmaz et al. 2014a; Avanus 2015). On the other hand, It was similar to some studies, which reported Hemşin sheep (84.2), Kangal sheep (0.84), and also 0.85 for the Karayaka sheep (Yilmaz et al. 2014a; Avanus 2015). Chung and Davis (2012) studied the ovine calpastatin genotypes and their association with body weight traits in sheep. Researchers reported that the Calpastatin genotypes affected birth weight and average daily live weight gain (Chung and Davis 2012). Khan et al. (2012) reported in sheep that animals with the MN genotype showed higher weight gain than in other genotypes. Armstrong et al. (2018) have found that C/T variation on the CAST gene had significant effects on birth weight and growth rate in Texel sheep. On the other hand, significant associations may not be observed in some studies (Bayram et al. 2019; Kumar et al. 2016).

The Chi-square analysis was performed to show if the studied populations are in Hardy–Weinberg equilibrium (HWE). The Chi-square results showed that the studied populations were in HWE except Ordu. Karayaka sheep was in HWE being similar to another study (Avanus et al. 2015).

In the study, the observed heterozygosity (0.162) was higher than expected heterozygosity (0.150). Observed heterozygosity was found similar with Colombian creole hair sheep (0.154) (Montes et al. 2019) but lower than Bulgarian Merino sheep breed (0.53) (Sakova et al. 2020). The observed heterozygosity value was also lower than those reported for some Turkish sheep breeds (Sakız; 0.506, Kivircik; 0.235, Karacabey Merino; 0.262) but higher than Imroz breed (0.020) (Yilmaz et al. 2014a; Yilmaz 2014b). Similarly, expected heterozygosity was also lower than those (0.506, 0.266, 0.320) reported in these studies, but higher than the Imroz breed (0.020).

This study is the first report comparing the populations for the CAST gene in the Karayaka sheep breed. There was no study comparing sub-populations of a breed for the CAST gene in Turkey. The populations' genetic structure is changed by many factors such as population size, breeding method, gene flow and geographical barriers (Slatkin et al.1987; Troell et al. 2006; Charlesworth, 2009). When considering these genetic structure changes, further

investigations at population level should be done to test the associations of polymorphism with the traits of growth and meat quality in Karayaka and other Turkish sheep breeds.

CONCLUSION

The present study first revealed genetic variation of CAST gene at the population level in Karayaka sheep breed. The study partially showed that genetic structure can vary among different geographic locations. Therefore, the determination at the population level of genetic polymorphism on major genes for meat quality traits such as the CAST gene could be more helpful for genetic breeding programs and future studies.

CONFLICT OF INTEREST:

The authors state no conflict of interest

AUTHOR CONTRIBUTION

KK performed the laboratory analysis. KK and LM performed statistical analysis of data. All authors wrote the article and approved the final paper.

REFERENCES

Aksoy Y, Çiçek Ü, Şen U, Şirin E, Uğurlu M, Önenç A, Kuran M, Ulutaş, Z. 2019. Meat production characteristics of Turkish native breeds: II. meat quality, fatty acid, and cholesterol profile of lambs. Archives Animal Breeding. 62 (1): 41.

Ardicli S, Dincel D, Samli H, Balci F. 2017. Effects of polymorphisms at LEP, CAST,CAPN1,GHR,FABP4 and DGAT1 genes on fattening performance and carcass traits in Simmental bulls. Archiv fuer Tierzucht. 60 (2): 61.

Armstrong E, Ciappesoni G, Iriarte W, Da Silva C, Macedo F, Navajas EA, Brito G, Julian S, Gimeno D, Postiglioni, A. 2018. Novel genetic polymorphisms associated with carcass traits in grazing Texel sheep. Meat science 145: 202-208.

Avanus, K., Genetic Variability of CAST Gene in Native Sheep Breeds of Turkey. 2015. Kafkas Universitesi Veteriner Fakultesi Dergisi. 21 (6): 789-794.

Balcıoğlu MS, Karslı T, Şahin E, Ulutaş Z, Aksoy Y 2014. Determination of calpastatin (CAST) gene polimorphism in some native sheep breeds reared in Turkey by PCR-RFLP method. Tarim Bilimleri Dergisi. 20 (4): 427-433.

Bayram D, Akyüz B, Arslan K, Özdemir F, Aksel EG, Çinar MU 2019 . DGAT1, CAST and IGF-I Gene Polymorphisms in Akkaraman Lambs and Their Effects on Live Weights up to Weaning Age. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 25(1): 9-15.

Bozhilova-Sakova, M, Dimitrova I, Tzonev TI, Petrov N. 2020. Genotype frequencies in calpastatin (CAST) and callipyge (CLPG) genes in Northeast Bulgarian Merino sheep breed using PCR-RFLP method. Bulgarian Journal of Agricultural Science. 26 (2): 475-479.

Cam MA, Olfaz M, Garipoglu AV. 2007. Shearing male lambs in the cold season improves the carcass yield without affecting fattening performance. Animal science journal. 78 (3): 259-265.

Charlesworth B. Effective population size and patterns of molecular evolution and variation. Nature Reviews Genetics. 10(3): 195.

Chung H, Davis M. 2012. PCR-RFLP of the ovine calpastatin gene and itsassociation with growth. Asian J Anim Vet Adv. 7(8): 641-652.

Dincel D, Ardicli S, Soyudal B, Er M, Alpay F, ŞAMLI H, Balci F. 2015. Analysis of FecB, BMP15 and CAST Gene Mutations in Sakiz Sheep. Kafkas Universitesi Veteriner Fakultesi Dergisi. 21 (4): 483-488.

Donicer Montes V, Claudia Lenis V, Darwin Hernández H. 2019. Polymorphisms of the calpain and calpastatin genes in two populations of colombian creole sheep. Revista MVZ Córdoba. 24(1): 7113-7118.

Eftekhari Shahroudi F, Nassiry MR, Valizadh R, Heravi Moussavi A, Tahmoores Pour M, Ghiasi H. 2006. Genetic polymorphism at MTNR1A, CAST and CAPN loci in Iranian Karakul sheep. Iranian Journal of Biotechnology. 4 (2): 117-122.22.

Gabor M, Trakovická A, Miluchová M. 2009. Analysis of polymorphism of CAST gene and CLPG gene in sheep by PCR-RFLP method. Scientific Papers Animal Science and Biotechnologies. 42 (2): 470-476.

Gabor, M., TRAKOVICKÁ, A., & MILUCHOVÁ, M. (2009). Analysis of polymorphism of CAST gene and CLPG gene in sheep by PCR-RFLP method. Scientific Papers Animal Science and Biotechnologies, 42(2), 470-476.

Gao Y U, Zhang R, Hu X, Li N. 2007. Application of genomic technologies to the improvement of meat quality of farm animals. Meat science. 77 (1): 36-45.

Gharahveysi S, Abbasi HA, Irani M, Abdullahpour R, Mirhabibi S. 2012. Polymorphism investigation of calpastatin gene in Zel sheep population of Iran by PCR-RFLP method. African Journal of Biotechnology. 11(13): 3211-3214.

Greguła-Kania M, Gruszecki TM, Junkuszew A, Juszczuk-Kubiak E, Florek M. 2019. Association of CAST gene polymorphism with carcass value and meat quality in two synthetic lines of sheep. Meat science. 154: 69-74.

Grochowska E, Borys B, Lisiak D, Mroczkowski S. 2019. Genotypic and allelic effects of the myostatin gene (MSTN) on carcass, meat quality, and biometric traits in Colored Polish Merino sheep. Meat science. 151; 4-17.

Khan S, Riaz MN, Ghaffar A, Ullah MF. 2012. Calpastatin (CAST) gene polymorphism and its association with average daily weight gain in Balkhi and Kajli sheep and Beetal goat breeds. Pakistan J Zool. 44 (2): 377-382, 2012.

Khederzadeh S. 2011. Polymorphism of calpastatin gene in crossbreedDalagh sheep using PCR-RFLP. Afr J Biotechnol. 10 (53): 10839-10841.

Kulikova K, Yuldashbaev Y, Hatataev S. 2018. The polymorphism of Cast and GDF9 genes in the Tuvan short-fat-tailed sheep population. Scientific Papers-Series D-Animal Science. 61 (1):14-17.

Kumar SN, Jayashankar MR, Chandrashekhar M, Vageesh P, Nagaraja R, Maniu GU. 2016. Association of genetic polymorphisms of Calpain and Calpastatin genes with growth traits in Bandur sheep. Indian Journal of Animal Sciences. 86 (8): 907-912.

Nanekarani S, Asadi N, Khederzadeh S. 2011. Genotypic frequency of Calpastatin gene in Lori sheep by PCR-RFLP method. Anim. Sci. 72: 606-620.

Nikmard, M, Molaee V, Eskandarinasab MP, Dinparast Djadid N, Vajhi AR. 2012. Calpastatin polymorphism in Afshari sheep and its possible correlation with growth and carcass traits. Journal of Applied Animal Research. 40 (4): 346-350.

Oğan, M. 2000. Effects of shearing on fattening performance of Karayaka male lambs. Veteriner Fakültesi Dergisi, Uludağ Üniversitesi. 19 (1/2): 25-27.

Olfaz M, Ocak N, Erener G, Cam MA, Garipoglu A V. 2005. Growth, carcass and meat characteristics of Karayaka growing rams fed sugar beet pulp, partially substituting for grass hay as forage. Meat Science. 70 (1): 7-14.

Palmer BR, Roberts N, Hickford JG, Bickerstaffe R. 1998. Rapid communication: PCR-RFLP for MspI and NcoI in the ovine calpastatin gene. J. Anim. Sci. 76 (5): 1499-1500.

Pomitun I, Rossokha V, Boyko Y, Guzevatyi O, Shpilka M, Kulibaba R. 2019. Analysis of calpastatin and callipyge genes polymorphism in Prydniprovska meat sheep. Agricultural Science and Practice. 6 (2): 58-65.

Slatkin M. 1987. Gene flow and the geographic structure of natural populations. Science. 236(4803):787-792.

Suleman, M., et al., Calpastatin (CAST) gene polymorphism in Kajli, Lohi and Thalli sheep breeds. African Journal of Biotechnology, 2012. **11**(47): p. 10655-10660.

Sun Y, Luo W, Xie H, Zhang Y, Cai H. 2015. Analysis of association between polymorphism of TFB2M gene and meat quality, growth and slaughter traits in Guizhou White Goat, a well-known Chinese indigenous goat breed. Pakistan Journal of Zoology. 47 (6): 1605-1610.

Szkudlarek-Kowalczyk, ME. 2011. Wiśniewska, and S. Mroczkowski, Polymorphisms of calpastatin gene in sheep. Journal of Central European Agriculture. **12**(3): 425-432.

Troell K, Engström A, Morrison DA, Mattsson JG, Höglund J. 2006. Global patterns reveal strong population structure in Haemonchus contortus, a nematode parasite of domesticated ruminants. International journal for parasitology. 36 (12): 1305-1316.

TUIK (Turkish Statistical Institute), available at: http://www.tuik.gov.tr/UstMenu.do?metod=temelist(last access: 13 July 2020), 2019.

Yeh FC, Yang R, Boyle TJ, Ye Z, Xiyan JM. PopGene32, Microsoft Windows-based freeware for population genetic analysis, version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada, 2000.

Yilmaz O, Cemal I, Karaca O, Ata N 2014b. Association of Calpastatin (CAST) gene polymorphism with weaning weight and ultrasonic measurements of loin eye muscle in Kıvırcık lambs. Journal of the Faculty of VeterinaryMedicine, Kafkas University. 20 (5): 675-680.

Yilmaz O, Sezenler T, Ata N, Yaman Y, Cemal I, Karaca O. 2014a. Polymorphism of the ovine calpastatin gene in some Turkish sheep breeds. Turkish Journal of Veterinary and Animal Sciences, 38(4), 354-357.

Zhang CY, Wang Z, Bruce HL, Janz J, Goddard E, Moore S, Plastow GS. 2014. Associations between single nucleotide polymorphisms in 33 candidate genes and meat quality traits in commercial pigs. Animal genetics. 45 (4): 508-516.