Calpastatin (CAST) Gene Polymorphism in Tsigai and Merinoland Sheep Breeds Under Conditions of the Republic of Crimea

Veronika Uppe*, *Tatiana* Kuevda, *Denis* Zubochenko, *Elena* Usmanova, and *Pavel* Ostapchuk

Federal State Budget Scientific Institution "Research Institute of Agriculture of Crimea", Simferopol, Republic of Crimea, Russian Federation

Abstract. The article presents the results of studying the Calpastatin (CAST) gene polymorphism in Tsigai (n = 23) and Merinoland sheep breed (n = 13). The purpose of the research. The possibility of using PCR-RFLP analysis to detect the Calpastatin (CAST) gene polymorphism in Tsigai and Merinoland sheep was the primary objective of this ongoing study. Research methods. Calpastatin (CAST) gene polymorphism was analyzed by PCR-RFLP analysis using the MspI endonuclease restriction. Results. We revealed the diversity of genotypes and allelic variants of the Calpastatin (CAST) gene in Tsigai and Merinoland Breeds. It was found that M allele of the Calpastatin locus is the most common. Frequencies of MM, MN and NN genotypes were found to be 74, 4 and 22 % in Tsigai breed. Among the representatives of Merinoland sheep, the frequencies of MM and MN genotypes were 92 % and 8%, respectively. No animals with NN genotypes were found.

1 Introduction

It has been known the tenderness depends largely on the amount of connective tissue, myofibrillar protein degradation, and intramuscular fat content. The tenderness of the meat is largely determined by genetic factors of the livestock itself. One of the genetic componen with an effect on meat tenderness is gene calpastatin [1, 2].

Calpastatin is a regulatory protein, highly specific endogenous μ- and m-calpains inhibitor (calcium-dependent neutral proteinase), which has no effect on other proteases (papain, B and D cathepsins, bromelain, trypsin, chymotrypsin, pepsin, plasmin, etc.). This calpains inhibitor was first identified in muscle. The name, calpastatin, was first proposed in 1979 by Takashi Murachi [3-6]. Calpastatin gene polymorphism determination in meat sheepbreeding is a crucial factor among molecular genetic markers [7].

Calpastatin prevents Calpain autoproteolytic activation, translocation across membranes and expression of catalytic activity. Inhibitor protein, Calpastatin, together with proteinase, Calpain, form a calcium-dependent proteolytic system found in all mammalian cells. According to A. Douillard et al., Calpastatin seems to be present only in vertebrate tissues;

* Corresponding author: veronikauppe925@gmail.com

the genomic sequences of Drosophila, C. elegans and Saccharomyces cerevisiae do not contain a calpastatin sequence. The calpain-calpastatin system is one of four proteolytic systems that play a significant role in skeletal muscle myofibrillar protein degradation indicating their active role in muscle growth. The reduction levels of the muscle proteolysis by elevating the calpastatin activity (or reduce the calpain activity) leads to a reduction in protein production and protein turnover. This stimulates animals' growth due to an effective muscle increase. However, even though the above-mentioned processes may be desirable for a live animal, the unintended consequence may be a decrease in postmortem proteolysis [8-10].

Calpastatin is a thermostable protein (at 100 ºC), resistant to dissociation when exposed to denaturing agents (urea, trichloroacetic acid, SDS) or pH variations, but susceptible to proteolytic degradation. The activity of Calpains depends on several factors such as calcium ion concentration of (Ca2+), autolysis, intracellular localization, phosphorylation. Presently, eight types of calpastatin isoforms are identified (I, II, III, IV, etc.) [8, 11].

The CAST gene in sheep is localized in chromosome 5 and has a total size of 89553 pairs of nucleotides. Polymorphism in the CAST gene was first described in 1998 on Dorset Down sheep breed using PCR-RFLP analysis. Based on the results, desired MN genotype had been identified in high productive animals.

Bearing in mind general properties and substrate specificity, Calpastatin (CAST) gene is viewed as a marker for meat tenderness and growth traits [12-14].

The objective of this study was to identify the possibility of using PCR-RFLP analysis to detect the Calpastatin (CAST) gene polymorphism in Tsigai and Merinoland sheep breeds.

2 Material and methods of research

The study was carried out in the FSBSI "Research Institute of Agriculture of Crimea" in the Laboratory of Molecular Genetics, Proteomics and Bioinformatics. Biological materials for DNA genotyping: venous blood samples collected into EDTA Vacuum Blood Collection Tube, which were collected from Tsigai (n=23) and Merinoland (n=13) sheep breeds. These samples were collected from different private farms in the Republic of Crimea. Genomic DNA was extracted from blood cells using "Isogene Diatom DNA Prep 100" kit of reagents. CAST gene fragment was identified using the following primer pair: CAST F: 5'-TTGTCATCAGACTTCACCT; CAST R: 5'-TCTTCTTTTCTCTTTGGGTGGA. Amplification was performed using PCR thermocyclers "Bio-Rad T100 Termal Cycler". Amplification protocol started at 94 °C (5 min) followed by 33 cycles of 94 °C (45 s), 60 °C (45 s) and 72 °C (45 s). Following the PCR amplification, further processing of the amplicons was performed by incubated mode at 37 °C for 3 h using MspI endonuclease restriction. Band patterns were viewed on 2% agarose gel stained with EtBr electrophoresis. The genotype was identified by determining the number and size of DNA restrictive fragments. The marker of the molecular weight of DNA "100+ bp DNA Ladder" ("Eurogen", Moscow) was used. PCR analysis was interpreted using the "TotalLab" programme.

3 Research results

While carrying out a molecular typing of the Tsigai and Merinoland sheep breeds, Calpastatin (CAST) allelic variants were identified (Figure 1).

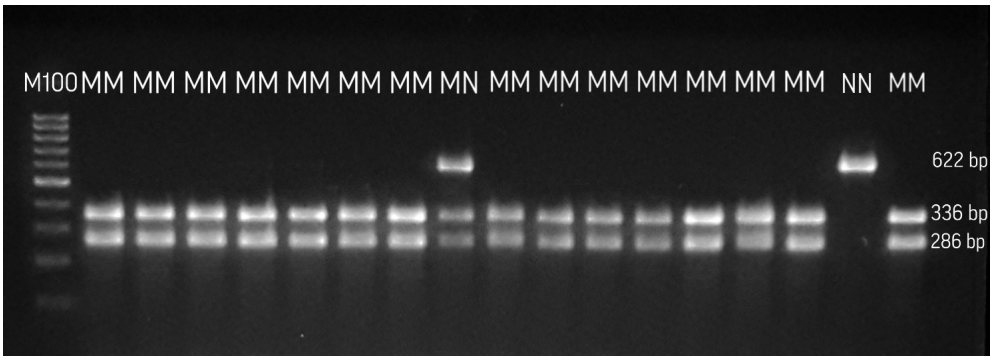

Fig. 1. Restriction Analysis of PCR Product of CAST Gene with MspI Restriction Enzyme on 2% Agarose Gel Electrophoresis in Tsigai and Merinoland sheep breeds (genotypes MM – 336, 286 bp; MN – 622, 336, 286 bp, NN – 622 bp; molecular weight of DNA "100+ bp DNA Ladder" ("Eurogen", Moscow).

CAST gene mutation 287A>C determines gene polymorphism. Three genotypes: MM (336bp and 286bp), NN (622bp) and MN (622bp, 336bp and 286bp) were identified after MspI digestion [6, 14].

The allele and genotype frequencies were calculated according to the standard methods [15]. They are presented in Table 1.

Table 1. Genotype and allele frequencies of the CAST gene of Tsigai and Merinoland Sheep Breeds.

Breed	Genotype	Number of animals with this genotype	Genotype frequency (%)	Allele frequency	
				M	N
Tsigai sheep (n = 15)	MM	17	74	0.85	0.15
	NN	1	4		
	MN	5	22		
Merinoland sheep (n = 13)	MM	12	92	0.97	0.03
	NN	0	0		
	MN	1	8		

As can be seen from the indicators mentioned above, most representatives of the Tsigai and Merinoland sheep breeds are of the MM genotype. We observed frequencies of 0.74, 0.04 and 0.22 of the MM, NN and MN genotypes in Tsigai sheep; 0.92, 0 and 0.08 of the same genotypes in Merinoland sheep, respectively. The allelic frequencies were observed to be 0.85 for the M and 0.15 for the N allele in Tsigai sheep; 0.97 and 0.03 in Merinoland sheep, respectively.

The search for the desired NN genotype in sheep breeding with interbreeding was carried out. The MM genotype occurrence frequency was in 30% of cases was established. Also, and MN-genotype 70% of cases. But NN genotype was not detected [12]. The MM, MN, and NN genotypes occurrence frequency in sheep of the Altai breed was 0.23, 0.72, and 0.05, respectively [16].

M genotype responsible for the formation of the keratin protein, which is branched off for the quality indicators of wool. This genotype was observed in almost all wool-producing sheep in India [17].

Sheep and goats tested for Myostatin content have predominantly alleles M and m. MM genotype with one at 337 bp; mm genotype at 125, 118, and 94 bp; Mm-genotype with four fragments at 337, 125, 118, and 94 bp [18].

The frequencies of MM, MN, and NN genotypes were 77-68, 20-26, and 3-6% in African sheep. Only the MM (80%) and MN (20%) genotypes were found in Tully sheep [19].

The presence of two genotypes M, MT with a frequency of 88 and 12%, respectively, was found in the Volgograd breed of sheep. The M allele and the homozygous MM genotype had the highest frequency [20].

Since growth hormone in prenatal development significantly affects muscle and bone formation, its level of presence in the genotypes of New Zealand Romney and Merino sheep breeds was established. The exon of growth hormone revealed multiple variations in the studied genotypes [21]. Thus, the study of this issue in the future still opens up wide prospects for researchers

3 Results discussion

In this study, the molecular-genetic investigations were carried out with PCR-RFLP analysis using the MspI endonuclease restriction. In the course of the research, we revealed the polymorphism in the CAST gene of Tsigai and Merinoland sheep breeds. As a result of this study, we found that two alleles (M and N) and three genotypes (MM, NN and MN) were presented in high frequencies in the studied Crimean Tsigai and Merinoland sheep breed. On the basis of the results obtained, it can be concluded that the MM genotype and the M allele are the dominant ones. Frequencies of M allele and MM genotype were found to be 85 and 74% in Tsigai breed and 97 and 92% in Merinoland breed, respectively.

4 Conclusions

The data obtained demonstrated the variety of the CAST genotypes and allelic variants of Tsigai and Merinoland sheep breeds from the Republic of Crimea. Also, this research results will allow researching relationships between Calpastatin gene polymorphism, growth performance and meat characteristics of Tsigai and Merinoland sheep breeds, particularly in the context of meat quality.

Acknowledgment

This work was supported by the State Program f FSBSI “Research Institute of Agriculture of Crimea” (State Registration Number 0562-2019-0001).

References

1. J.D. Leal-Gutiérrez, M. A. Elzo, D. D. Johnson, T.L. Scheffler, J.M. Scheffler, R.G. Mateescu, Association of μ-Calpain and Calpastatin Polymorphisms with Meat Tenderness in a Brahman–Angus Population, **1-10** (2018)
2. C. Sumantri, R. Diyono, A. Farajallah, I. Inounu, Calpastatin Gene Polymorphism (CAST-Msp1) and Its Effect on Live Weight of Local Sheep, **13**(2), 117-126 (2008)

3. J.D. Leal-Gutiérrez, M.A. Elzo, D.D. Johnson, T.L. Scheffler, J.M. Scheffler, R.G. Mateescu, Front Genet. J. Rr., **1–9** (2018)
4. M Farhadian, A Hashemi, K Mardani, R Darvishzadeh, M Ranjbari J. Agr. Sci. **10**(50), 10083-10086 (2011)
5. O. Yilmaz, I. Cemal, O. Karaca, N. Ata, Kafkas Univ Vet Fak Derg, **20** (5), 675-680 (2014)
6. D.Y. Degtyarev, L.N. Skorykh, D.V. Kovalenko, S.A. Emelyanov, N.V. Konik, RJPBCS, **7**(4), 2137-2139 (2016)
7. M.I. Selionova, L.N. Chizhova, E.S. Surzhikova, N.A. Podkorytov, A.T. Podkorytov, T.V. Voblikova, IOP Conf. Series: Earth and Environmental Science **613,** 012130 (2020)
8. N. Sorimachi, S. Hata, Y. Ono J. Proc Jpn Acad Ser B Phys Biol Sci, 287–327 (2011)
9. Melloni, E. Salamino, F. Sparatore, Bianca, Biochimie, 217–223 (1992)
10. M. Aali, M. Moradi-Shahrbabak, H. Moradi-Shahrbabak, M. Sadeghi, Gene. J. **1**, 537(1), 132-9 (2014)
11. A. Douillard, O. Galbes, G. Begue, V. Rossano, J. Levin, V. Vernus, A. Bonnieu, R. Candau, G. Py Clin Exp Pharmacol Physiol., **39**(4), 364-72 (2012)
12. B.R. Palmer, J.D. Morton, N. Roberts, M.A. Ilian, R. Bickerstaffe, Proc. N.Z. Soc. Anim. Prod. **59**, 266-268 (1999)
13. V. Pogodaev, B. Aduchiev, L. Kononova, M.Aslanukova, I. Kardanova, E3S Web of Conferences, **03020** (2020)
14. G.N. Serdyuk, A.O. Prituzhalova, J. Ovcy, kozy, sherstyanoe delo, **2,** 10–12 (2019)
15. I.A. Pomitun, O.A. Bojko, L.V. Shulika, I.V. Korh, R.O. Kulibaba, L.I. Pomitun Naukovo tekhnichnij byulleten' ITNAAN, **118**, 148–153 (2017).
16. K.A. Kulikova Vestnik Bashkir State Agrarian University, **1** (45), 84–89 (2018)
17. R. Kumar, A.S. Meena, R. Kumari, B. Jyotsana, L.L.L. Prince, S. Kumar. Indian Journal of Small Ruminants **22**(1), 28–31 (2016)
18. O.E. Othman, E.A. Balabel, E.R. Mahfouz, Biotechnology, **15** (1-2), 44-51 (2016)
19. M. Suleman, S. U. Khan, M. N. Riaz, M. Yousaf, A. Shah, R. Ishaq, A. Ghafoor. African Journal of Biotechnology, **11**(47), 10655-10660 (2012)
20. Yu. A. Kolosov, I. F. Gorlov, A. Yu. Kolosov, N. V. Shirokova, A. Ya. Kulikova, M. A. Kolosova, M. I. Slozhenkina, E. S. Vorontsova, N. N. Kolosova, IOP Conf. Series: Earth and Environmental Science, **677,** 052112 (2021)
21. U.J. Ekegbu, I.L. Haruna, G. Mahmoud, H. Zhou, J.G.H Hickford.World Journal of Agriculture and Soil Science **1**(2) (2018)