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CAST/MspI gene polymorphism and its impact on growth traits of Soviet Merino and Salsk sheep breeds in the South European part of Russia

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Abstract: The purpose of this work was to study *MspI* polymorphism in the *CAST* gene in Soviet Merino and Salsk sheep breeds in the South European part of Russia and to find its relation with growth traits. Soviet Merino sheep have been found to have the M and N alleles with a frequency of 0.88 and 0.12 and the MM, MN, and NN genotypes with a frequency of 0.82, 0.12, and 0.06, respectively. Salsk sheep have been established to have the M and N alleles with a frequency of 0.89 and 0.11 and two genotypes, MM and MN, with a frequency of 0.78 and 0.22, respectively. A significant relationship between the *CAST* genotypes and the growth traits of Salsk sheep has been revealed. The absence of the homozygous NN genotype in Soviet Merino sheep did not allow us to determine what exactly causes the effect of increasing the average daily gain of sheep – the presence of the N allelic variant or the combination of the heterozygous M and N alleles. The results obtained show the *CAST/MspI* gene to be promising as a marker of sheep production in developing sheep breeding programs to improve fattening and meat qualities.

Key words: Sheep, Salsk breed, Merino breed, calpastatin gene, *CAST*, marker assisted selection

1. Introduction

Sheep raising in the Russian Federation is a specialized branch of animal husbandry with a rich gene pool and has about 40 breeds and breed groups (1). As a branch of livestock production, sheep breeding certainly leads in terms of variety of products and is a priority development branch of agriculture in the south of Russia. The Salsk sheep breed was developed in the South European part of Russia over the course of about 20 years (1930–1950). The stock breeding of a new fine-wooled sheep breed involved breeding animals with a sound constitution, without defects and exterior deficiencies, long-wooled and well adapted to grazing conditions, capable of long travel, and able to make full use of the sparse vegetation in the Salsk steppes (2).

Currently, the Salsk sheep breed is well adapted to the specific living conditions of the Salsk steppes. The animals have a sound constitution. The ram's grease fleece production is 14–17 kg (5–9 kg of clean fleece weight) and the ewe's grease fleece production is 5–8 kg (2.5–3.0 kg of clean fleece weight). The clean equivalent weight is 47%–50%. The wool from gimmers and tegs is mostly 20.6–23.0 μm, and it is 23.1–25.0 μm from stud rams, 80–90 mm long, and white. The live weight of rams is 95–110 kg, and for ewes it is 50–55 kg. The birth rate of the Salsk sheep is 124%–140% (3).

The Soviet Merino is a fine-wooled sheep breed with a high yield of high-quality fleece and meat. It is grown throughout Russia on large and small farms. The Soviet Merino gains weight well and fattens on a diet of concentrate feed with mineral additives and fresh grass. Rams have strong, massive horns, which help to easily identify the Soviet Merinos on any pasture (4). A mature ewe can reach up to 98 kg of weight and a ram up to 150 kg. The slaughter yield is 42%–48%.

The Soviet Merino looks very typical; it can be considered the standard for a wool sheep. It has a large body, folds of skin on the front of the face, massive horns in males, and a longitudinal fold at the bottom of the neck. The wool is thick, which makes the body of the animal look like a barrel. On its head, the wool grows everywhere except the nose, eyes, and mouth.

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Nowadays, however, one of the economically important breeding objectives for the Soviet Merino is to improve the fattening performance and meat quality along with the wool productivity. To achieve positive results in this field, development and implementation of the marker selection (marker-assisted selection, MAS) becomes important as it provides for the use of DNA markers associated with the level of production character manifestation (5–7). The genes, allelic variants of which are associated with the phenotypic expression of economically relevant traits of the animals (weight, height, meat yield, etc.), are considered as the DNA markers (8–10).

Today, the calpastatin gene (*CAST*) is tested as a promising marker of meat productivity of sheep. Calpastatin is an endogenous highly specific inhibitor, a powerful regulator of the calpain activity in the cell. Calpain is present in virtually all cells and tissues of vertebrates in various isoforms. Formation of an active calpain–calpastatin complex in the presence of calcium ions has a significant impact on cellular function and provides the regulation of essential life processes, which are synaptic transmission, secretion, cell differentiation, metabolism of muscle proteins, and many others (11,12).

The *CAST* gene (Gene ID: 443364) in sheep is localized on chromosome 5; it consists of 29 exons and has an overall size of 89,553 bp. The gene polymorphism located in the first intron between exons 1C and 1D may be determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method using the restriction endonuclease *MspI* (13). Investigations conducted to study the polymorphism in different sheep breeds showed a statistically significant association between the *CAST* genotypes and growth and weight traits of sheep (14,15).

Thus, technologies based on DNA markers are widely applied in national breeding programs in several countries with a developed sheep-raising industry and have significant impact on improving the composition of the carcass, the meat quality, and the efficiency of mutton production. Development of methods for more efficient use of the available gene pool breeds of sheep, reduction of feed costs, application of genetic control systems, the finding of additional reserves, and improvement of the economic performance of the industry is the most important task of sheep breeding at the present stage of development in Russia and in the world. Given the relevance of molecular–genetic studies, both abroad and in the majority of Russian breeding farms, the importance of evaluating the candidate genes responsible for the productive qualities of sheep is undeniable. The study of the *CAST/MspI* gene polymorphism in sheep will increase the accuracy of the estimate of breeding value of animals bred in Russia and in other countries.

2. Materials and methods

The purpose of our work was to study the *MspI* polymorphism in the *CAST* gene (*CAST/MspI*) in sheep bred in the South European part of Russia and to find any relationship between the *CAST* genotypes and growth traits. The investigations were carried out on sheep of the Soviet Merino (n = 72) and Salsk (n = 108) breeds in the Southern Federal District (LLC Belozernoye in the Salsk district of Rostov Province), Russia. To carry out the molecular genetic studies, sample tissues of 1 cm^2 were taken from the ear area of the animals. DNA was isolated using a kit of reagents, DIAtom DNA Prep 100 (LLC Research and Production Company Genlab, Russia). Analysis was performed by PCR-RFLP.

To amplify a fragment of the *CAST* gene 622 bp long (exon 1C: 61 bp, intron 1: 473 bp, and exon 1D: 88 bp), these primers were used:

Ovine 1C: 5′-TGGGGCCCAATGACGCCATCGATG-3′

Ovine 1D: 5′-GGTGGAGCAGCACTTCTGATCACC-3′

The following protocol was applied: predenaturation at 95 °C for 4 min and then 35 cycles of 94 °C for 45 s, 62 °C for 45 s, and 72 °C for 45 s, with final synthesis performed at 72 °C for 7 min (13). Restriction of the amplified fragment was performed by endonuclease *MspI*. In the presence of the restriction site, two fragments, 336 and 286 bp long, were formed that corresponded to the M allele; in the absence of the site, the fragment length remained unchanged (622 bp). The size of the restriction fragments was determined by electrophoresis in 2% agarose gel in the presence of ethidium bromide.

The presence and the frequency of alleles and genotypes were established according to the results of the molecular genetic analysis (16). The allelic and genotypic frequencies, the heterozygosity observed (Ho) and expected (He), and the Hardy–Weinberg equilibrium test were calculated using PopGene 3.1 software. The frequency of genotypes was determined by the formula $p = \frac{n}{N}$, where p is the frequency of the genotype determination, n is the number of individuals with a specific genotype, and N is the number of individuals. The frequency of certain alleles was determined by the formula $PA = \frac{(2nAA + nAB)}{2N}$, $qB = \frac{(2nBB + nAB)}{2N}$, where PA is the frequency of allele A, qB is the frequency of allele B, and N is the total number of alleles. The expected results of the genotype frequencies in the studied population were calculated by the Hardy–Weinberg law.

The influence of the *CAST* genotypes on the growth traits of rams was investigated according to the following factors: birth weight (kg), weight at weaning (kg) and at the age of 2 months, and average daily gain of the animal from birth to 2 months of age (g). All test animals were of the same year of birth and were kept in the same housing conditions.

The data on different variables, obtained from the experiment, were statistically analyzed by the Statistica 10 package (StatSoft Inc., USA). The significance of differences between the indices was determined using the criteria of nonparametric statistics for the linked populations (differences with $P < 0.05$ were considered significant: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns = not significant at $P > 0.05$). Student's t-test was applied for the statistical analysis (17,18).

The mean of a set of measurements was calculated according to the formula $\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$, where $\bar{x}$ is a mean value, $\sum_{i=1}^{n} x_i$ is read as "the sum of all x_i with i ranging from 1 to n", and n is the number of measurements. The residual variation is expressed as a root mean square error (RMSE): $\sigma = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}}$. The standard error of the mean (*s.e.m.*) was calculated by the formula $s.e.m.(\bar{x}) = \frac{\sigma}{\sqrt{n}}$. The reliability of a sample difference (Student's t-distribution) was estimated by the test of the difference validity, which is the ratio between the sample difference to the nonsampling error. The test of the difference validity was determined by the formula $t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s.e.m._1^2 - s.e.m._2^2}} \geq t_{st}(d.f. = n_1 + n_2 - 2)$, where t is Student's t-distribution, $(\bar{x}_1 - \bar{x}_2)$ is the difference of the sample mean measurements, $\sqrt{s.e.m._1^2 - s.e.m._2^2}$ is the sample difference error, $s.e.m._1$ and $s.e.m._2$ are the nonsampling errors of the sample statistics compared, t_{st} is the standard criterion according to the t-table for the probability threshold preset depending on degrees of freedom, n_1 and n_2 are the numbers of measurements in the samples compared; and *d.f.* is the degrees of freedom for the difference of two mean measurements.

Microsoft Office 2010 was employed for graphical presentation of the data.

3. Results

The results of the molecular genetic analysis revealed the presence of the *CAST/MspI* polymorphism in Soviet Merino and Salsk sheep breeds. The allelic *CAST/MspI* variants presented by the fragments of 622 bp (the N allele) and of 336 and 286 bp (the M allele) were present in both sheep breeds studied, as shown in Figure 1.

The Soviet Merino sheep were shown to have 3 genotypes, MM, MN, and NN, with a frequency of 0.82, 0.12, and 0.06, respectively (Table 1). The Salsk sheep were found to have only 2 genotypes, MM and MN, with a frequency of 0.78 and 0.22, respectively. In general, the homozygous MM genotype had the highest frequency in the sheep breeds analyzed. The homozygous NN genotype was found only in the Soviet Merino breed, but the frequency of the heterozygous MN genotype in this breed was much lower compared with the Salsk breed.

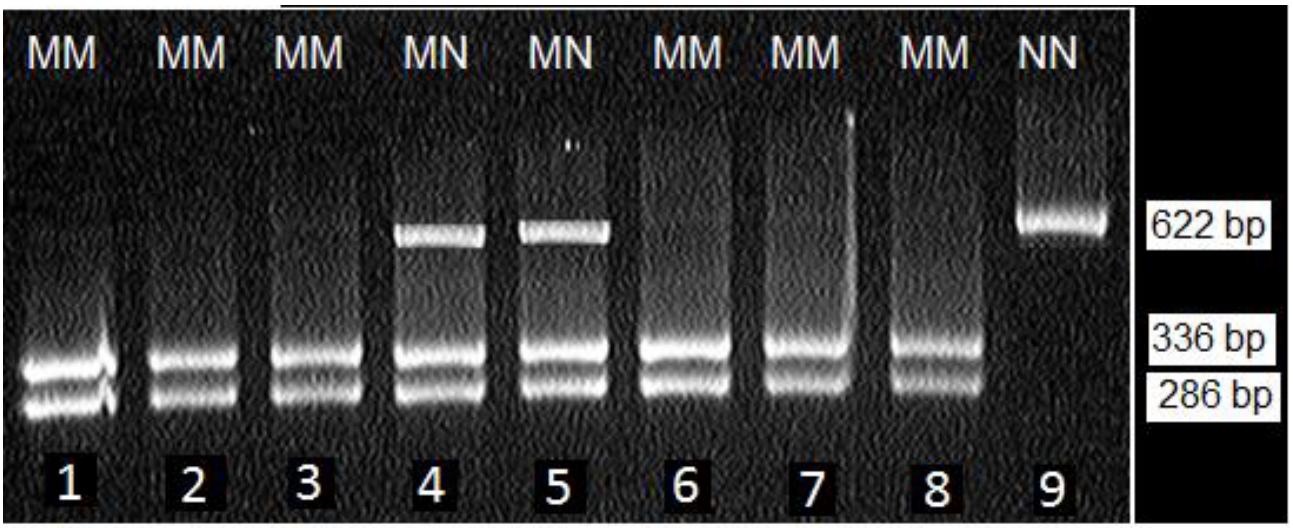

Figure 1. Electropherogram of the PCR-RFLP analysis of the *CAST* gene for *MspI* in the Salsk breed (lanes 1–4) and the Soviet Merino (lanes 5–9). The sizes of the *MspI* gene fragments are shown on the right side.

Table 1. The allele and genotype frequencies for the *CAST/MspI* gene in Soviet Merino and Salsk sheep breeds.

Breed	Allelic frequency		Genotype frequency		
	M	N	MM	MN	NN
Soviet Merino (n = 72)	0.88	0.12	0.82	0.12	0.06
Salsk (n = 108)	0.89	0.11	0.78	0.22	-

4. Discussion

The metaanalysis of published data on 22 sheep breeds in Poland, Iran, Pakistan, Turkey, Indonesia, and other countries showed the presence of the *CAST/MspI* polymorphism in all breeds (Table 2). The homozygous MM genotype, with the frequency varying from 0.90 to 0.47 depending on the breed, was prevalent in almost all breeds, except Harri, Lori, Arkhamerino, and Mehraban. In sheep breeds Harri, Lori, and Arkhamerino, the heterozygous MN genotype had the highest frequency, and only the Mehraban breed was seen to have the homozygous NN genotype at the highest rate. The results found by Asadi et al. showed that in the population of Lori sheep genotypes MM, MN, and NN occurred at frequencies of 32.2, 63.2, and 4.6, respectively (19). Georgieva et al. in 2015 found that genotype frequencies of the Synthetic Population Bulgarian Milk sheep breed were 0.84, 0.15, and 0.01 for MM, MN, and NN in the calpastatin locus, respectively (20).

The M allele with the frequency of 0.33 to 0.99, depending on the breed, was prevalent in all breeds except Arkhamerino and Mehraban. In the study sample of sheep breeds, the N allele frequency was registered in a range from 0.01 to 0.67. Figure 2 shows an ordered

Table 2. The allele and *CAST/MspI* genotype frequency in various sheep breeds.

No.	Breed	n	Frequency					Ref.*
			Allelic		Genotype			
			M	N	MM	MN	NN	
1	Polish Merino	82	0.76	0.24	0.56	0.40	0.04	(21)
2	Berrichon du Cher	41	0.92	0.08	0.85	0.15	-	
3	Blackheaded Mutton	59	0.81	0.19	0.71	0.20	0.09	
4	Ile de France	30	0.95	0.05	0.90	0.10	-	
5	Bandur	79	0.74	0.26	0.55	0.39	0.06	(15)
6	Jastru	264	0.86	0.14	0.75	0.23	0.02	(9)
7	Najdi	20	0.66	0.34	0.64	0.36	-	(23)
8	Harri	20	0.87	0.13	0.26	0.74	-	
9	Lori	48	0.64	0.36	0.41	0.46	0.13	(19)
10	Balkhi	300	0.88	0.12	0.76	0.24	-	(14)
11	Kajli	300	0.86	0.14	0.74	0.24	0.02	
13	Shumen	121	0.92	0.08	0.84	0.15	0.01	(20)
14	Gökçeada	49	0.99	0.01	0.98	0.02	-	(7)
15	Kıvırcık	336	0.85	0.15	0.73	0.24	0.03	
16	Karacabey Merino	248	0.80	0.20	0.67	0.26	0.07	
17	Sanjabi	98	0.72	0.28	0.54	0.37	0.09	(22)
18	Afshari	30	0.63	0.37	0.54	0.17	0.29	
19	Ghezel	65	0.69	0.31	0.47	0.25	0.28	
20	Makui	32	0.88	0.12	0.82	0.12	0.06	
21	Arkhamerino	42	0.48	0.52	0.28	0.47	0.25	
22	Mehraban	25	0.33	0.67	0.17	0.37	0.46	

* Ref. = Reference.

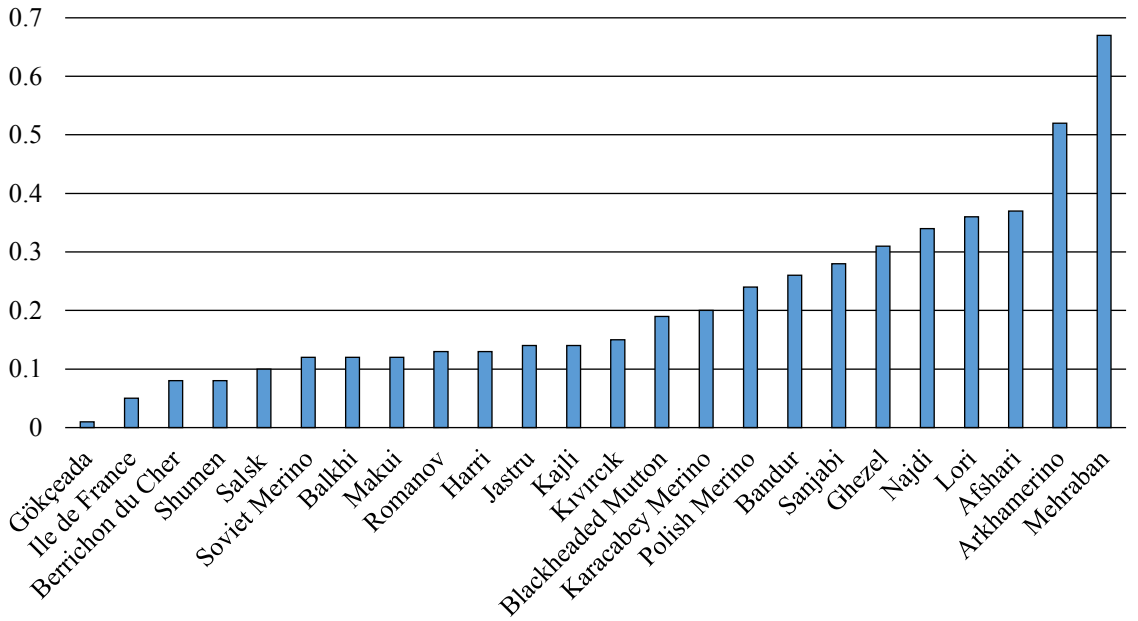

Figure 2. Distribution of the N allele of the *CAST/MspI* gene in various sheep breeds.

series of the N allele frequencies in different sheep breeds. It is interesting to note that the greatest N allele frequencies (0.28–0.67) are characteristic of Sanjabi, Ghezel, Lori, Afshari, Arkhamerino, and Mehraban, which are bred in Iran, as well as Najdi in Turkey. The lowest N allele frequencies (0.01–0.11) were registered in Gökçeada (Turkey), Ile de France and Berrichon du Cher (Poland), Shumen (Bulgaria), and Soviet Merino (Russia). Szkudlarek-Kowalczyk et al. in 2011 found that the Polish Merino sheep had the M and N alleles with a frequency of 0.76 and 0.24 and the MM, MN, and NN genotypes with a frequency of 0.56, 0.40, and 0.04, respectively (21). Tohidi et al. found that the M allele in Shanjabi, Afsahri, Ghezel, and Mehraban sheep breeds was more predominant than other alleles at this locus. The highest to lowest observed heterozygosity values were 0.47 (Arkhamerino), 0.37 (Sanjabi), 0.25 (Ghezel), 0.17 (Afshari), and 0.12 (Makui) (22). Yılmaz et al. found that the allele frequencies for M and N alleles of the gene were 0.85 and 0.15 in Kıvırcık, 0.80 and 0.20 in Karacabey Merino, 0.99 and 0.01 in Gökçeada, and 0.34 and 0.66 in Sakız sheep, respectively. It was determined that the NN genotype frequency was lower in Kıvırcık (0.04) and Karacabey Merino (0.07) populations than in the Sakız breed (0.40). On the other hand, the NN genotype was not observed in the Gökçeada population (7). Saleha and Alakilli found that frequencies of allele M were 0.66 and 0.87, while they were 0.34 and 0.13 for allele N in Najdi and Harri breeds in Saudi Arabia, respectively (23).

Further research examining the relationship between the allelic variants of the *CAST* gene and the growth traits showed that the presence of the heterozygous MN genotype in rams of the Salsk breed resulted in greater daily gain, by 16.3 g, and greater weight at weaning at the age of 2 months, by 1.04 kg (Table 3).

Similar results were obtained by Khan et al. (14). According to their study, the presence of a heterozygous MN genotype resulted in greater daily gain in the period from birth to 8 months of age in sheep of the Balkhi breed and from birth to 4 months in Kajli sheep. The paper by Tahmoorespour et al. (24) also presented a positive effect of the heterozygous genotype on the average daily gains of Kurdi and Baluchi sheep. In our research, the Salsk sheep had a heterozygous genotype, which resulted in greater daily gain from birth to weaning at the age of 2 months.

Thus, for the first time, the *CAST/MspI* gene polymorphism in sheep breeds in the Southern Federal District of Russia has been observed and a significant relationship between the *CAST* genotypes and the growth traits of the Salsk sheep breed has been revealed. The studied sheep population of the Soviet Merino breed has been established to have only 2 genotypes (MM and MN). The absence of the homozygous NN genotype did not allow us to determine what exactly causes the effect of increasing the average daily gain of sheep, the presence of the N allelic variant or the combination of the heterozygous M and N alleles. However, the results obtained show the *CAST/MspI* gene to be promising as a marker of sheep production, and further research in this area will allow for reliable estimating of the impact of allelic variants of the

Table 3. Growth traits of the Salsk sheep breed according to different genotypes of the *CAST/MspI* gene.

Genotypes	Weight at birth, kg	Weight at weaning, kg	Average daily gain, g
MM (n = 84)	4.11 ± 0.07	22.19 ± 0.27	301.12 ± 5.79
NM (n = 24)	4.19 ± 0.18	23.23 ± 0.38*	317.43 ± 2.01**

* P < 0.05; ** P < 0.01.

gene on the growth traits and for the development of sheep breeding programs that take into account the *CAST/MspI* polymorphism to improve the fattening and meat qualities of sheep.

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