MC4R gene polymorphism and its association with meat traits of Karachai sheep grown in Russian Federation

Ivan Fiodorovich Gorlov ab*, Nadezhda Vasilievna Shirokova ab*, Elena Yuriyevna Anisimova abe**, Marina Ivanovna Slozhkenina ab*, Yury Anatolyevich Kolosov cae, Anatoliy Yuriyevich Kolosov c, Natalia Ivanovna Mosolova ab, Maria Anatolyevna Kolosova c, Timur Tazretovich Tarchokov fl**, Aleksandr Anatolyevich Mosolov a, Daria Aleksandrovna Mosolova a, and Ekaterina Vladimirovna Karpenko a, e

Volga Region Research Institute of Manufacture and Processing of Meat-And-Milk Production, Rokosovskogo Street, 6, Volgograd 400013, Russia; bVolgograd State Technical University, Volgograd, Russian Federation; cDon State Agrarian University, Laboratory of Molecular Diagnostics and Biotechnology of Farm Animals, Persianovsky, Russian Federation; dKalmyk State University, Elista, Russian Federation; eVolgograd State University, Volgograd, Russian Federation; fKabardino-Balkarian State Agrarian University named after V.M. Kokov, Nalchik, Russian Federation; gPlekhanov Russian University of Economics, Moscow, Russian Federation

ABSTRACT
This paper presents the results on the study of melanocortin-4 receptor (MC4R) gene polymorphisms in sheep of the Karachai breed. AA, AG, and GG genotypes were determined to have allele frequencies of 47%, 37%, and 16%, respectively, indicating that allele A and the homozygous AA genotype occurred the most frequently. Analysis of the meat productivity of sheep, with respect to the MC4R genotype, showed a higher slaughter yield in sheep with the AA genotype compared to those with either the GG or AG genotypes. The variability at these loci provides a basis for controlling the meat productivity of sheep in the Karachai sheep population; therefore, this polymorphism should be monitored and melanocortin-4 receptor (MC4R) gene selection considered in pedigree farms.

Introduction

The problem of pedigree resources needing to be improved and rationally used in sheep breeding remains an urgent problem that requires modern approaches to be solved (Rasali et al. 2006; Yurchenko et al. 2019). Traditional breeding methods are successfully used to improve parameters that are economically important for farms and large agricultural enterprises (growth rate, feed conversion, intramuscular fat content, meat yield, etc.) (Shumbusho et al. 2016). All of these parameters are relatively easy to measure in living animals or in controlled slaughter; however, the effect of selection according to these parameters is not always significant due to rather wide variation in the level of heritability among different herds. Under the influence of heritability, a certain characteristic metabolism is formed in animals which creates the final effect of productivity. However, as a sum of many genetically regulated processes, metabolism is still under the influence of the external environment. Its level and nature are influenced by environmental, feed, hierarchical, and other paratyphical factors. Therefore, greater objectivity in the conclusions on an animal’s genetic potential can be achieved using data obtained through immunogenetic analysis methods and genetic markers in conjunction with biochemical and ethnological studies (Callaghan and Beh 1996).

In addition to the economically useful parameters above, requirements for the quality of raw meat have also become stricter (Benoit et al. 2019). Producers receive significant economic benefits from an increase in slaughter weight combined with high meat yield and reduction in feed costs per unit of production. Consumers are interested in improving meat qualities such as its juiciness, tenderness, taste, and colour (Lescheva and Ivolga 2015). Traditional methods to improve the quality of mutton including breeding as well as improving feeding and meat processing technology (Buschulte et al. 2005).

Over the past decade, advances in molecular genetics and biology have made it possible to decipher sequences of the sheep genome, which has opened up interesting opportunities for the study and understanding of genetic factors that influence the quality of lamb meat (Deniskova et al. 2016;...
These new tools are of particular importance as they can be used to assess the potential of an animal immediately after birth and do not require their slaughter (Zinovieva et al. 2015). Several of the basic functional polymorphisms used in practical breeding affect post-mortem parameters, such as posthumous pH, colour, and tenderness, of meat have been identified. Some candidate genes include loci of quantitative parameters associated with economically useful traits, and they have allowed us to evaluate the genetic potential of animal productivity (Gorlov et al. 2016; 2017; 2018). Evaluation of the animal genotype has facilitated the identification and accumulation of preferred alleles in the population. At the same time, the spectrum of DNA markers associated with reproductive, meat, and fattening traits is constantly expanding.

Among the candidate signalling molecules involved in the regulation of energy homeostasis and that also affect the qualitative parameters of meat productivity, the melanocortin-4 receptor (MC4R, GenID: 100147707) is of particular interest. A point mutation in the seventh exon of the MC4R gene disrupts leptin hormone signalling through MC4R and, thus, affects the traits that determine the fattening and meat productivity of the animal (Bruun et al. 2006; Jokubka et al. 2006; Klimenko et al. 2014). The search for and identification of DNA markers associated with productivity traits of farm animals are of particular relevance due to the intensification of activity in the livestock industries (Rasali et al. 2006; Shum-busho et al. 2016). One of the main genes that determines the development of these productive traits in sheep is the melanocortin 4 receptor gene (MC4R). MC4R is one of the five currently identified melanocortin receptors that belong to the G-protein receptor family and encodes a seven-domain transmembrane protein. MC4R is expressed in the central nervous system (thalamus, hypothalamus, spinal cord, and cortex), mainly in the hypothalamic region, and it regulates feed intake and energy balance. Although information on the relationship between the MC4R polymorphism and productive traits of sheep is ambiguous, rather noticeable effects of this polymorphism on the values for average daily gain, feed intake, muscle growth, and carcass fat have been found (Getmantseva et al. 2014). In the vast majority of studies, the ratio between the MC4R genotypes, in terms of the growth rate, was AA > GG. In some studies, an inverse relationship (AA < GG) was found, or no dependencies between the MC4R genotypes and the level of development of this trait were revealed. Thus, the influence of the MC4R genotype manifests differently depending on the breed of sheep.

According to the published data, the biological function of MC4R is in the control of eating behaviour (Kim et al. 2000). In the melanocortin-4 receptor gene, a mutation has been found that causes sheep to eat more (about 10%), grow faster (68%), and have increased live weight gain (6% – 10%) (Getmantseva et al. 2014). The aim of our work is to confirm that this polymorphism melanocortin-4 receptor (MC4R) gene is also found in Karachai sheep bred in Russia and to determine its effect on the productive parameters of this sheep breed.

Materials and methods

Animals and housing

The study was conducted in the territory of the Karachay region in 2018–2019 (Figure 1). The main Karachai sheep breeding base is located in the Karachay-Cherkess Republic, where there are 5 breeding plants and 4 breeding reproducers with 111,200 sheep, including 85,400 purebred ewes. The total number of Karachai sheep in ‘Dargan’ today is 8400. The main breeding place for Karachai sheep is in the North Caucasus mountain zone (i.e. the territories of the republics of

Figure 1. Sheep of the Karachai breed.
Kabardino-Balkaria and Karachay-Cherkessia). Karachai sheep are one of the oldest fat-tailed sheep, and they have a unique exterior. Along with the Lysgin and Tushino breeds, Karachai meat, wool, and dairy productivities are well developed, with herds of Karachai sheep used for either wool or meat production (Kolosov et al. 2013).

Of great demand is the meat of Karachai sheep, characterized by fine fibre, moderate fat deposition in the muscles, excellent taste, and high nutritional benefits. The weight of internal and subcutaneous fat reaches 40% of the carcass weight of an adult animal.

The sheep are notable for their medium size, but they have a relatively long body and voluminous chest. Sheep of this breed are hardy, mobile, and well adapted to grazing, including in highland and lowland pastures, and easily tolerate transitions over long distances. These characteristics are provided by their physiological features and solid hooves. The tail at the base is rounded or lyre-shaped; the tip, without fat deposits, is curved in the form of the letter S and ends at the level of the hock joints.

All experimental livestock were grown in accordance with the regulations for keeping animals, as established in the ‘Dargan’ (Kabardino-Balkarian Republic, Russian Federation).

Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Guide for the care and use of laboratory animals, 2011). The use of experimental animals was in accordance with local animal welfare laws and policies. The current study is in compliance with ethical standards, and the sheep owner provided written consent for the use of his animals in this study. All studied animals had minimal age differences, sharing the same birth year in addition to having the same feeding conditions and daily routine, and were served by the same employees.

In this research work, data on the primary breeding livestock were registered, and the results of our own research (assessment of the productive traits of parents, control slaughter conducted, selection, and laboratory studies of biomaterial) were analyzed. The test group of animals (n = 427) included unrelated male offspring obtained from 35 rams that were 3 years of age and 920 ewes that were 2 years of age. All parental combinations belonged to the Karachai sheep breed. When organizing reproduction, panmixia was observed. This excluded the influence of directional selection of parental combinations. The age difference of young animals in the test group did not exceed 4 days. Complete siblings were not included in the test groups.

**Sample collection and Genomic DNA isolation**

Samples (1 cm²) were taken from an auricle of the Karachai sheep (n = 427) (ear notch). DNA was isolated from the ear notches using the DIAtom DNA Prep 100 reagent kit ('Research and Production Company GenLab', D1024, Russia) in accordance with the manufacturer's instructions.

**PCR analysis**

PCR was performed on a Tercik amplifier, Russia.

| Table 1. MC4R gene locations. |
|-----------------------------|
| **MC4R gene Ovis Aries**    |
| Chromosome                  | 23                      |
| Chromosome position         |                         |
| Beginning of gene           |                         |
| End of gene                 |                         |
| Gene length                 |                         |
| Position of SNP studied     |                         |
| Position                    |                         |
| Replacement                 |                         |
| Primers                     |                         |
| F Primer Position           | 1173–1196 bp            |
| F Primer Length             | 24 bp                   |
| R Primer Position           | 1424–1446 bp            |
| Primer length               | 23 bp                   |
| PCR Product Length          | 274 bp                  |

The **MC4R** gene has a total length of 3869 bp. The region of amplification was from 1173 to 1446 bp (Table 1).

The composition of the PCR mixture for amplification (Tersus Plus PCR kit, PK121, Evrogen, Russia) was as follows (in a final reaction volume of 25 μL per each sample): 5× Tersus Red buffer; 3.0 mM Mg²⁺; 20 pmol of each oligonucleotide primer (forward and reverse, Table 1); 0.2 mM of dNTP mixture; 1.0 units (U) of Taq DNA Polymerase; about 50–500 ng of isolated DNA; and nuclease-free, sterile, double-distilled water up to 25 μL.

The **MC4R** fragment gene was amplified in the following order: initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 54.8–66 °C for 30s, and final extension at 72 °C for 10 min.

**SNP genotyping**

The amplified **MC4R** gene fragment was restricted using AcⅠI endonuclease (‘SibEnzyme-M’, Russia). The recognition site was C↓TGCG; GGC↑G. For restriction analysis of the obtained PCR products, 6 μL PCR product, 2.5 μL ddH₂O, 0.5 μL restriction enzyme (1 U), and 1 μL enzyme buffer were mixed in a final volume of 10 μL. Hydrolysis was carried out at a temperature of 35 °C for 1 h in a TT-2 Termit thermostat (‘Research and Production Company DNA-Technology’, Russia). Fragment sizes were determined in comparison with a 100 bp molecular weight marker (10 fragments of from 100 to 1000 bp, SibEnzyme, Russia, 1 μg per lane), supplied with 1 mL of 6× DNA stain by electrophoresis in 2.5% agarose gel (‘Syntol’, Russia), and stained with ethidium bromide (‘Syntol’, Russia). The restriction fragments obtained were visualized in ultraviolet light.

**Genetic diversity analysis**

Molecular genetic analysis was used to determine the presence and frequency of alleles and genotypes (Nei and Kumar 2000). 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. This research allowed us to solve the problem of assessing the state of the sheep populations under study in terms of the statistical significance of differences in the values of the expected and observed heterozygosity. The frequency of...
heterozygotes is an important indicator, since each heterozygous individual carries different alleles and, thus, indicates the presence of variability.

**Slaughter traits**

Meat qualities with respect to the following parameters: pre-slaughter weight (kg), fresh carcass weight (kg), chilled carcass weight (kg), weight of internal fat (kg), weight of tail fat (kg), slaughter weight with tail fat and without tail fat (kg), and slaughter yield with tail fat and without tail fat (%) in 4-month-old rams that were subject to controlled slaughter in accordance with the requirements of GOST 31777–2012 ‘Sheep and goats for slaughtering. Mutton, lambs and goats in carcasses. Specifications’.

**Statistical analysis**

Two linear mixed models (LMMs) were used:

1. A basic model that included two effects—flock effect (fixed) and additive animal genotype effect (randomized); and
2. A test model that additionally involved the MC4R genotype factor (fixed).

The models were then compared using one-way analysis of variance (ANOVA) to determine the significance of differences between the two models.

Based on the comparison results and taking into account the fact that, from a structural point of view, the difference lies in the presence of the MC4R genotype factor in the test model, we concluded that the influence of this factor on productivity was significant.

The data on different variables, obtained from the experiment, were statistically analyzed by Statistica 10 package (StatSoft Inc.). The significance of differences between the indices was determined using the criteria of nonparametric statistics for linked populations (differences with \( P < 0.05 \) were considered significant; NS = not significant at \( P > 0.05 \)). Student’s t-test was applied for the statistical analysis (Johnson and Bhattacharyya 2010) Table 2.

**Results and Discussion**

**Results**

The fragments of a certain length obtained during the first stage of analysis and resulting from the restriction analysis using the AcI restriction endonuclease allowed us to identify the A and G allelic variants of the MC4R gene and determine the possible genotypes present in the studied sheep stock (Figure 2).

The results of DNA testing of the melanocortin-4 receptor (MC4R) gene for the presence of the A and G allelic variants and possible genotypes using the PCR-RFLP method in Karachai sheep are presented in Table 3.

Thus, in sheep of the Karachai breed, three genotypes, AA, AG, and GG, were found at frequencies of 47%, 37%, and 16%, respectively. The data in Table 2 show that the A allelic variant of the melanocortin-4 receptor (MC4R) gene was the most widely found in the studied population.

Expected and observed heterozygosity values in the Karachai sheep population under study are presented in Table 4.

Our work found no significant difference between expected and observed heterozygosity. According to the \( \chi^2 \) value (at a significance level of \( P < 0.01 \)) obtained, the distribution of observed heterozygous genotypes reliably corresponded to that expected according to the Hardy–Weinberg equilibrium principle, which indicated the studied populations were in genetic equilibrium.

Further studies on the relationship between the MC4R allelic variants and the growth rate showed that the homozygous AA genotype in Karachai sheep was positively associated with the growth rate of young animals. The average daily gains in the AA

![Figure 2](Image)

**Figure 2.** PCR-RFLP electrophoretic analysis of the MC4R gene in Karachai sheep: AA genotype (226 bps); GG genotype (156 and 70 bps); AG genotype (226, 156, and 70 bps); Marker (100 bps).
genotype sheep were also higher by 12.7 g (P < 0.05) and 18.7 g (P < 0.001), respectively, compared to their peers with the AG and GG genotypes (Table 5).

The relationship between the allelic variants of the MC4R gene and the meat productivity of Karachai sheep was further studied, and the best meat productivity was observed in rams with the AA/MC4R genotype, significantly exceeding that observed in rams of either the AG/MC4R and GG/MC4R genotypes in almost all of the analyzed traits (Table 6).

Analysis of the samples obtained using controlled slaughter indicates that Karachai sheep with the AA/MC4R genotype were superior to rams with the AG/MC4R and GG/MC4R genotypes by 1.5 (ns) and 2.2 kg (P < 0.05), respectively, in terms of the pre-slaughter weight. The slaughter weight of rams with the AA/MC4R genotype also exceeded that of rams with the AG/MC4R and GG/MC4R genotypes by 1.4% (P < 0.05) and 2.4% (P < 0.001), respectively. The chilled carcass weight for the AA/MC4R genotype was higher than that of AG/MC4R and GG/MC4R animals by 0.9% (P < 0.05) and 1.6% (P < 0.001), respectively.

### Discussion

Along with traditional methods of selection, genotype selection can considerably increase the efficiency of improving both the livestock of an individual farm and the breed as a whole. The melanocortin-4 (MC4R) gene polymorphism in Karachai sheep has not previously been studied. This research is important for both the science and practice of the sheep industry.

Against the background of the limited implementation of genetic study results, the search for polymorphic variants of the melanocortin-4 receptor (MC4R) gene that are potential markers for sheep meat productivity is an extremely urgent task for the breed under consideration.

In studying the melanocortin-4 receptor (MC4R) gene polymorphism according to the method described above, a greater distribution of the A allele compared to the G allele was found in representatives of various breeds. Also, the superiority of the AG genotype over the AA and GG genotypes, in terms of its frequency of occurrence, has been repeatedly noted in other studies. Thus, the results obtained in our study were consistent with the results of similar works performed on other sheep breeds, including indigenous breeds.

The conducted work enabled us to solve the problem of assessing the state of the studied sheep populations from the point of view of the reliability of differences between the expected and observed heterozygosity. The frequency of heterozygotes is an important indicator because each heterozygous individual possesses different alleles and, thereby, illustrates variability.

The MC4R gene has been studied in various mammals, including pigs (Kim et al. 2000; 2004; Houston et al. 2004; Stachowiak et al. 2005), cattle (Liu et al. 2012; Huang and Wang 2013), and sheep (Song et al. 2012).

According to the study of the MC4R gene in pigs of the Large White breed, Klimenko et al. (2014) noted that individuals with the homozygous AA genotype were characterized by the best average daily gain and greater thickness of bacon compared to animals with the GG genotype. Similar results were obtained by Leonova (2013), where three genotypes, AA, AG, and GG, were found in Landrace pigs at frequencies of 19%, 46.6%, and 34%, respectively. A positive effect of the homozygous AA genotype on average daily gains and meat productivity of animals was established. An assessment on the influence of genotypes on the fattening and meat traits of the Lekss pig line (born in 2010) found that, compared to pigs with the AA and GG genotypes of the MC4R gene, the AG genotype animals were notable for their better early maturity, on average, by 4.9 days (3%, P < 0.05); daily average growth, by 68.5 g (8.4%, P < 0.05); and lower fat, by 0.23 mm (1.8%, P < 0.05); but they were slightly inferior in terms of the carcass length. Similar patterns were observed in Lekss pigs born in 2013. The AG genotype of MC4R, compared to the AA and GG genotypes, was associated with a better average maturity by 6.3 days (3.8%, P < 0.05), daily average gain by 54.4 g (7.4%, P < 0.05), body length by 1.1 cm (0.87%), and lower fat by 1.1 mm (7.4%, P < 0.05).

### Table 3. The frequency of alleles and genotypes of the MC4R gene of Karachai sheep, n = 427.

| Gene | Alleles | Genotypes, % |
|------|---------|--------------|
|      | A       | G            |
| MC4R | (n = 201)| (n = 158)    |
|      | 0.65    | 0.35         |
|      | 47      | 37           |
|      | 16      |              |

### Table 4. Observed and expected heterozygosity values of the MC4R gene of Karachai sheep.

| Number of animals (n) | Heterozygosity observed (H0) | Heterozygosity expected (H0) | \( \chi^2 \) |
|-----------------------|-----------------------------|-----------------------------|----------|
| 427                   | 0.372                       | 0.455                       | 1.537    |

### Table 5. Growth dynamics of Karachai sheep according to MC4R genotype.

| Traits                      | GG (n = 68) | AG (n = 158) | AA (n = 201) |
|-----------------------------|------------|-------------|-------------|
| Live weight at birth, kg:   | 3.51 ± 0.06 | 3.60 ± 0.13 | 3.57 ± 0.09 |
| Live weight at the age of 4 months, kg: | 32.9 ± 0.59 | 33.6 ± 0.77 | 35.1 ± 0.87 |
| Average daily gain, g       | 244.0 ± 4.39 | 250.0 ± 5.04 | 262.7 ± 3.56 |

Note: A = P < 0.001, C = P < 0.05, NS = not significant at P > 0.05 compared with data on the AA genotype.

### Table 6. Measured parameters of Karachai sheep that underwent controlled slaughter according to different genotypes, mean ± SEM.

| Parameters                        | Groups of animals with genotype |
|-----------------------------------|---------------------------------|
|                                  | GG (n = 68) | AG (n = 158) | AA (n = 201) |
| Pre slaughter weight, kg          | 32.9 ± 0.59 | 33.6 ± 0.77 | 35.1 ± 0.87 |
| Weight of hot carcass, kg         | 14.8 ± 0.40 | 15.5 ± 0.41 | 16.3 ± 0.34 |
| Weight of chilled carcass, kg     | 14.4 ± 0.22 | 15.1 ± 0.38 | 16.0 ± 0.24 |
| Weight of intramuscular fat, kg   | 0.4 ± 0.11  | 0.5 ± 0.11  | 0.7 ± 0.20  |
| Weight of tail fat, kg            | 0.9 ± 0.13  | 1.1 ± 0.09  | 1.4 ± 0.12  |
| Slaughter weight, kg: with tail fat | 15.7 ± 0.42 | 16.7 ± 0.36 | 18.1 ± 0.44 |
| without tail fat                  | 14.8 ± 0.32 | 15.5 ± 0.31 | 16.7 ± 0.40 |
| Slaughter yield, % with tail fat  | 47.7        | 49.7        | 51.6        |
| without tail fat                  | 45.0        | 46.4        | 49.5        |

Note: A = P < 0.001, B = P < 0.01, C = P < 0.05, NS = not significant at P > 0.05 compared with data on the AA genotype.
0.01). However, Park et al. (2002) did not find major effects on fatness with the Large White × wild boar intercross.

In the literature, there are sufficient data from studies conducted on the MC4R polymorphism variant in different sheep breeds and according to different productivity directions.

DNA diagnostics of the MC4R gene polymorphism carried out on Chinese Hu sheep (Liu et al. 2012) showed that animals with the GG genotype were distinguished by their lean meat. The AA genotype sheep had the highest live weight at weaning, highest average daily gain from birth to weaning, as well as the highest pre-slaughter weight and meat productivity.

Zuo et al. (2014) investigated the MC4R gene polymorphism in German Merino sheep. According to their results, on the 120th and 180th days, the AA genotype sheep had higher average daily gains than their peers with the AG and GG genotypes.

Getmantseva et al. (2014) presented data on the distribution of MC4R genotypes among sheep of the Volgograd breed. Three genotypes were established, AA, AG, and GG, with frequencies of occurrence of 12%, 59.4%, and 28.6, respectively. In general, the G allele and the heterozygous AG genotype had the highest frequency in the Volgograd sheep breed. The obtained results show that sheep of the AA genotype reliably outperform their GG genotype counterparts in terms of early maturity (1.65 days, \( P < 0.05 \)) and daily average gain (by 110 g, \( P < 0.01 \)).

The SNP positions in different animal species are not identical and MC4R is found on different chromosomes in different animal species.

Conclusions

Our work revealed that the MC4R gene polymorphism is also found in sheep of the Karachai breed and we further characterized its effect on meat traits, which made it possible to determine desired genotypes for further breeding to increase the specific weight of sheep in this population. The results of the study demonstrate that it is advisable to use genetic markers to optimize sheep breeding programmes in order to increase the profitability of mutton production. To optimize and monitor sheep breeding processes, decisions must be made regarding how to improve the Karachai sheep breed based on the determination of genetic status and specific available breeding resources. In this context, it is advisable to use data on the frequencies of MC4R genotypes as markers of polymorphisms. Selecting these markers during sheep breeding will help increase the breeding value to the desired level through the identification of desired genotypes in the animal population.

Acknowledgments

We are very thankful to the staff of the Open Joint Stock Company ‘Dargan’ (Kabardino-Balkarian Republic, Russian Federation) for their support and help during the entire experiment period. We are also grateful to the Russian Science Foundation for the financial support of this research (grant number 19-76-10010 NIIMMP).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the [Russian Science Foundation] under Grant [19-76-10010 NIIMMP].

Animal Welfare Statement

All experimental livestock were grown in accordance with the regulations for keeping animals, as established in the ‘Dargan’ (Kabardino-Balkarian Republic, Russian Federation). Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Guide for the care and use of laboratory animals, 2011). The use of experimental animals was in accordance with local animal welfare laws and policies. The current study is in compliance with ethical standards, and the sheep owner provided written consent for the use of his animals in this study. The authors declare that animal tissue samples were collected by trained personnel under strict veterinary rules. Sampling was performed in accordance with the ethical guidelines of the L.K. Ernst Federal Science Center for Animal Husbandry.

Data Availability

All data generated or analysed during this study are included in this published article. The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

ORCID

Ivan Fiodorovich Gorlov http://orcid.org/0000-0002-8683-8159
Nadezhda Vasilevna Shirskova http://orcid.org/0000-0002-5607-3044
Elena Yuriyevna Anisimova http://orcid.org/0000-0002-7508-3897
Marina Ivanovna Slozhhenkina http://orcid.org/0000-0001-9542-5893
Yury Anatolevich Kolosov http://orcid.org/0000-0002-6826-8009
Anatolyi Yuriyevich Kolosov http://orcid.org/0000-0002-6583-8942
Natalia Ivanovna Mosolova http://orcid.org/0000-0001-6559-6595
Timur Tazretovich Tarchakov http://orcid.org/0000-0002-7434-1700
Alessandr Anatolevich Mosolov http://orcid.org/0000-0002-3266-9505
Daria Aleksandrovna Mosolova http://orcid.org/0000-0002-5579-6726
Ekaterina Vladimirovna Karpenko http://orcid.org/0000-0003-6463-6431

References

Benoit M, Sabatier R, Lasseur J, Creighton P, Dumont B. 2019. Optimising economic and environmental performances of sheep-meat farms does not fully fit with the meat industry demands. Agron Sustain Dev. 39 (4):40.

Brunn CS, Jorgensen CB, Nielsen VH, Andersson L, Fredholm M. 2006. Evaluation of the porcine melanocortin 4 receptor (MC4R) gene as a positional candidate for a fatness QTL in a cross between Landrace and Hampshire. Anim Genet. 37(4):359–362.

Buschulte A, Bachari M, Fries R. 2005. The sheep: A market under control? Fleischwirtschaft. 85:97–101.

Callaghan MJ, Beh KJ. 1996. Genetic markers as selection criteria. In Sustainable Parasite Control in Small Ruminants. ACIAR Proceedings Series (ed. by LeJambre, L.F.; Knox, M.R.). Proceedings Paper International Workshop on Sustainable Parasite Control in Small Ruminants, Bogor, Indonesia, 74, 178–185.
Committee for the Update of the Guide for the Care and Use of Laboratory Animals; Institute for Laboratory Animal Research (ILAR); Division on Earth and Life Studies (DELS); National Research Council of the national academies. 2011. Guide for the care and use of laboratory animals, 8th ed. Washington: The National Academies Press.

Denisova TE, Dotsev AV, Selionova MI, Kunz E, Medugorac I, Reyer H, Wimmers K, Barbato M, Traspop AA, Brem G, Zinovieva NA. 2016. Comparative analysis of the effectiveness of STR and SNP markers for intraindividural and interspecific differentiation of the genus Ovis. Russ J Genet. 52:79–84.

Getmantseva LV, Tretyakova OL, Leonova MA. 2014. Practical use of MC4R gene polymorphism in sheep breeding. Scientific and practical recommendations (in Russian).

Gorlov IF, Kolosov YA, Shirokova NV, Getmantseva LV, Slozhkenkina MI, Mosolova NI, Bakoev NF, Leonova MA, Kolosov AY, Zlobina EY. 2017. Association of the growth hormone gene polymorphism with growth traits in Salsk sheep breed. Small Ruminant Res. 150:11–14.

Gorlov IF, Kolosov YA, Shirokova NV, Getmantseva LV, Slozhkenkina MI, Mosolova NI, Bakoev NF, Leonova MA, Kolosov AY, Zlobina EY. 2018. GDF9 gene polymorphism and its association with litter size in two Russian sheep breeds. Rendiconti Lincei-Scienze Fisiche E Naturali. 29:61–66.

Gorlov IF, Shirokova NV, Randelin AV, Voronkova VN, Mosolova NI, Zlobina EY, Kolosov YA, Bakoev NF, Leonova MA, Bakoev SY, et al. 2016. CAST/ mspl gene polymorphism and its impact on growth traits of Soviet Merino and Salsk sheep breeds in the South European part of Russia. Turkish J Vet Anim Sci. 40:399–405.

Houston RD, Cameron ND, Rance KA. 2004. A melanocortin-4 receptor (MC4R) polymorphism is associated with performance traits in divergently selected large white pig populations. Anim Genet. 35:386–390.

Huang YT, Wang MS. 2013. The influence of the integrated model of social stratification structure on the public participating non-profit organizations. J Soc Sci. 9:151–158.

Johnson RA, Bhattacharyya GK. 2010. Statistics Principles and methods, 6th ed. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Jokubka R, Maak S, Kerziene S, Swalve HH. 2006. Association of a melanocortin 4 receptor (MC4R) polymorphism with performance traits in Lithuanian White pigs. J Anim Breed Genet. 123:12–22.

Kim KS, Larsen NJ, Rothschild MF. 2000. Rapid communication: linkage and physical mapping of the porcine melanocortin-4 receptor (MC4R) gene. J Anim Sci. 78:791–792.

Kim KS, Reecy JM, Hsu WH, Anderson LL, Rothschild MF. 2004. Functional and phylogenetic analyses of a melanocortin-4 receptor mutation in domestic pigs. Domest Anim Endocrinol. 26:75–86.

Klimenko A, Usatov A, Getmantseva L, Kolosov Y, Tretyakova O, Bakoev S, Kostjunina O, Zinovieva N. 2014. Effect of melanocortin-4 receptor gene on growth and meat traits in pigs raised in Russia. Am J Agric Biol Sci. 9(2):232–237.

Kolosov Y, Getmantseva L, Shirockova N. 2013. Sheep breeding resources in Rostov Region. World Appl Sci J. 23:1322–1324.

Leonova MA, Kolosov AY, Ryadyuk AV, Bagel EM, Stetyukha AA, Svyatogorova AE. 2013. Promising gene markers of productivity of farm animals. Young Scientist [Molodoj Uchenyj]. 12(59):612–614. (in Russian).

Lescheva M, Ivolga A. 2015. Current state and perspectives of sheep breeding development in Russian modern economic conditions. Ekonomika Poljoprivreda-Econ Agric. 62:467–480.

Liu Q-Y, Yu Y-S, Jin X. 2012. Association analysis on polymorphisms of prolactin receptor (PRLR) gene exon 10 with reproductive traits in songliao black pig and landrace pig. J Chin Anim Husb Vet Med. 39 (10):191–195.

Nei M, Kumar S. 2000. Molecular evolution and phylogenetics. New York: Oxford University Press.

Park HB, Carlborg O, Marklund L, Andersson L. 2002. Melanocortin-4 receptor (MC4R) genotypes have no major effect on fatness in a large white x wild boar intercross. Anim Genet. 33:155–157.

Rasali DR, Shrestha JNB, Crow GH. 2006. Development of composite sheep breeds in the world: a review. Can J Anim Sci. 86:1–24.

Shumbusho F, Raoul J, Astruc JM, Palhierie I, Lemarie S, Fugeraay-Scarbel A, Elsen JM. 2016. Economic evaluation of genomic selection in small ruminants: a sheep meat breeding program. Animal. 10(6):1033–1041.

Song XM, Jiang JF, Zhang GZ, Shi FX, Jiang YQ. 2012. DNA polymorphisms of the Hu sheep melanocortin-4 receptor (MC4R) gene associated with birth weight and 45d-weaning weight. Genet Mol Res. 11(4):4432–4441.

Stachowiak M, Szydowski M, Obarzanek-Fojt M, Switonski M. 2005. An effect of a missense mutation in the porcine melanocortin-4 receptor (MC4R) gene on production traits in polish pig breeds is doubtful. Anim Genet. 37:55–57.

Yurchenko AA, Denisova TE, Yudin NS, Dotsev AV, Khamiruev TN, Selionova MI, Egorov SV, Reyer H, Wimmers K, Brem G, et al. 2019. High-density genotyping reveals signatures of selection related to acclimation and economically important traits in 15 local sheep breeds from Russia. BMC Genomics. 20(30):article #294.

Zinovieva NA, Selionova MI, Gladyr EA, Petrovic MP, Caro Petrovic V, Ruzic Muslic D, Petrovic MM. 2015. Investigation of gene pool and genealogical links between sheep breeds of Southern Russia by blood groups and DNA microsatellites. Genetika-Belgrade. 47:395–404.

Zuo BY, Liu GQ, Peng YQ, Qian HG, Liu JS, Jiang XP, Mara A. 2014. Melanocortin-4 receptor (MC4R) polymorphisms are associated with growth and meat quality traits in sheep. Mol Biol Rep. 41(10):6967–6974.