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# THE POLYMORPHISM OF *CAST* AND *GDF9* GENES IN THE TUVAN SHORT-FAT-TAILED SHEEP POPULATION

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***Abstract***

*The Tuvan short-fat-tailed sheep is a local breed spread in the Russian Federation. This breed habites in Tuva. The aim of investigation was the identification of the genetic polymorphism of calpastatin (CAST) and the growth differentiation factor-9 (GDF9) genes in the Tuvan local sheep population. Calpastatin gene is known as a candidate gene of meat quality traits, and GDF9 gene is a potential genetic marker of prolificacy. Genomic DNA was isolated from samples of blood of 131 animals. Two primer pairs were used to obtain 622 b.p. fragment of CAST gene and 462 b.p. fragment of GDF9 gene. Calpastatin locus was digested with MspI restriction enzyme. Two genotypes (MM and MN) of CAST gene were observed. The polymorphism of GDF9 gene (CC and CD genotypes) was detected after amplicons digestion with AspLEI restriction enzyme. MM and MN genotypes were identified with 0.855 and 0.145 frequencies, M and N allele frequencies were 0.928 and 0.072, respectively. In this population CC and CD genotypes of GDF9 gene were identified with 0.878 and 0.122 frequencies, C and D allele frequencies were 0.939 and 0.061, respectively.*

***Key words****: sheep, genetic polymorphism, CAST, GDF9, PCR-RFLP.*

## INTRODUCTION

The Tuva Republic is a one of the most important region of the Russian Federation characterized by conventional sheep breeding. That region is situated in southern Siberia, in the geographical center of Asia.

It's the region with alternation of mountain ranges and intermountain basins, characterized by steppe landscape. Mountains constitute over 80% of the region territory.

The climate of region is s sharply continental.

Sheep breeding is the main branch of agriculture of the Tuva Republic and the important part of traditional way of life of tuvans. The main target in the Republic is supplying of sheep meat.

The major indigenous sheep breed in that region is the Tuvan short-fat-tailed breed (Tuvan sheep breed).

Sheep of Tuvan breed are adapted to specific regional climate environment. Animals have a high immunity and stamina (Yuldashbaev et al., 2016).

The level of production traits of sheep depend on environment in each year and season.

The breed contains two breed types: steppe and mountain sheep, that are differ by level of productive traits. For example, the minimal live weight of adult rams of steppe type is 78 kg, of ewes - 56 kg. The natural wool is strong, 12-14 cm length. Adult rams of mountain type characterized by 55 kg of live weight, ewes - 42 kg. Length of wool is 10-12 cm.

The birth rate of the Tuvan sheep is 100-110 lambs per 100 ewes. The clean equivalent weight of wool is 50-60% (Yuldashbaev et al., 2016).

Sheep breeding in the Tuva Republic is extensive brunch of animal husbandry, expansion of production is achieved by increasing the total number of sheep and unlimited using of pasture.

Intensification of this brunch can be achieved by using methods which are increasing the level of production without extension of sheep and using maximum of resources from each sheep. The one of solution for intensification of sheep breeding is using DNA markers of productive traits for organization marker-assisted selection. Marker-assisted selection (MAS) is applying of DNA markers to improve

the response to selection in a population of animals. The markers should be closely linked to one or more target loci, which may often be quantitative trait loci.

One of the potential marker gene for growth traits and to improving meat tenderness after slaughter is ovine calpastatin gene (Deykin et al., 2016). Calpastatin gene (CAST) is of 100 kb length, includes four exons and is located on the fifth sheep chromosome (Palmer et al., 1998).

Growth differentiation factor-9 gene (GDF9), also situated on fifth sheep chromosome, knows as a potential genetic marker of prolificacy**.** This gene contain two exons and one introns (Bahrami et al., 2014).

Detection of polymorphic variants of genes is one of key moments of beginning of selection program**.** The polymorphic variant of gene, can be associated with different levels of productive traits.

The aim of this investigation is detection of polymorphism of CAST and GDF9 genes to estimate possibility of using these genes in selection programs.

## MATERIALS AND METHODS

PCR-RFLP method was the basis for detecting genes polymorphism.

Blood samples for DNA analyses were collected from 131 purebred rams of Tuvan short-fat-tailed breed, were raising for herd replacements. Animals were presented from municipal unitary enterprise "Chalaaty". K3-EDTA tubes were used for good safety of samples. Approximately 9.0 mL blood samples were collected in sterile tubes. All volumes with blood were frozen in -20ºC.

Genomic DNA was isolated from blood samples using the commercial kits as per the manufacturer's instructions.

The DNA amplification of CAST gene was achieved by using following pair of primers: CAST - F: 5'-TGGGGCCCAATGACGCCAT CGATG-3' and CAST R: 5'-GGTGGA GCAGCACTTCTGATCACC-3' (Palmer et al., 1998). The PCR was implemented by following parameters: a preliminary denaturizing at 95ºC for 3 min, followed by 1 cycle of denaturing at 95ºC for 15 sec, annealing at 60ºC for 40 sec, and extension at 72ºC for 30 sec, followed by 35 cycles. A final extension by 5 min at 72ºC. The PCR products of CAST gene were digested at 37°C for 12-16 hours with *MspI* restriction enzyme (Alakilli, 2015).

To obtain GDF9 gene amplicons was used following pair of primers: GDF9-F: 5'-GAAGACTGGTATGGGGAAATG-3';

GDF9-R: 5'-CCAATCTGCTCCTACACACC T-3'. The amplification reaction conditions were carried out using 35 cycles at 94°C for 2 min., followed by 94°C for 30 sec, 63°C for 40 sec, 72°C for 30 sec, and final extension at 72°C for 4 min (Kolosov et al., 2015). The obtained GDF9 CAST gene PCR products were digested at 37°C for 12-16 h with *AspLEI* restriction enzyme.

All amplicons and digested PCR products of CAST and GDF9 loci were separated in 2.0 - 3.0% agarose gel and visualized by ethidium bromide staining under gel documentation system.

The data generated by electrophoresis of *MspI* and *AspLEI* digested product of samples was used for estimating the frequency of different restriction fragment patterns.

The genotypes and allelic frequency were estimated by standard procedure (Falconer, 1989).

Genotypes frequency was calculated in following formula:

$$P_i = \frac{n_i}{N},$$

where: $P_i$ - i$^{th}$ genotype frequency;

$n_i$ - number of samples of i$^{th}$ genotype;

$N$ - total number of samples of individuals of all genotypes.

Allelic frequency was calculated by in the following way:

$$p_i = \frac{2n_{(homozygote)} + n_{(heterozygote)}}{2N},$$

where: $p_i$ - i$^{th}$ allele frequency;

$n$ - number of homozygotes of particular gene and heterozygotes, respectively;

$N$ - total number of individuals.

## RESULTS AND DISCUSSIONS

Amplified products of 622 b.p. fragment of CAST gene was obtained after amplification in all the analyzed samples (Figure 1).

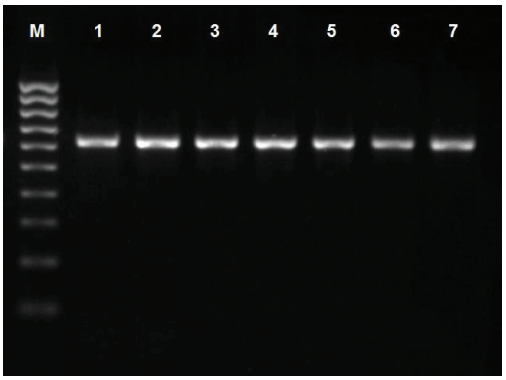


Figure 1. PCR products of CAST gene with size of 622 (lanes 1-7), lane M – 100 p.b. DNA ladder. Viewed on 2.0% agarose gel

N and M alleles of CAST gene were observed after the digestion of 622 b.p. PCR product with restriction enzyme. The *MspI* digestion of the amplicons produced fragments of 336 b.p. and 286 b.p. for allele M, and the allele N was not digested.

The homozygous genotypes MM were characterized by 336 b.p. and 286 b.p. bands. The heterozygous genotype MN was consist of 3 bands: 622 b.p., 336 b.p. and 286 b.p. NN genotype (622 b.p.) was absent in that population, but according the other literature sources It looks as a non-digested amplicon (Palmer et al., 1998; Tohidi et al., 2013).

PCR products of 462 b.p. fragment of GDF9 gene was obtained after amplification (Figure 2).

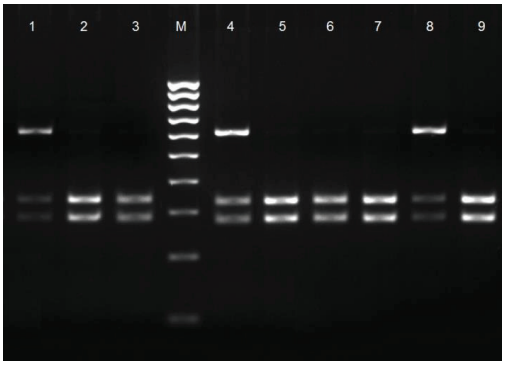


Figure 2. DNA electrophoretic pattern of CAST amplicons after digestion with *MspI* restriction enzyme: lane M - 100 p.b. DNA ladder, lanes 2, 3, 5, 6, 7, 9- MM genotype, lanes 1, 4, 8 - MN genotype. Viewed on 2.0% agarose gel

Alleles C and D of DGF9 gene were detect by digesting of PCR products by *AspLEI* restriction enzyme (Figure 3), then two genotypes presented by fragments of different size were established.

The *AspLEI* digestion of amplificated loci of gene produced fragments of 254, 156 and 52 b.p. for allele C, and the allele D was described like a 410 and 52 b.p. patterns.

The homozygous genotypes CC was characterized by 254, 156 and 52 b.p. bands. CD genotype was consist of 4 bands: 410, 254, 156 and 52 b.p. Fragments with size of 52 b.p. were bad visible (Figure 3).

DD genotype was not observed in the population. It contains 410 b.p. and 52 b.p fragments and has a low frequency, according the previous investigations (Bahrami et al., 2014; Kolosov et al., 2015).

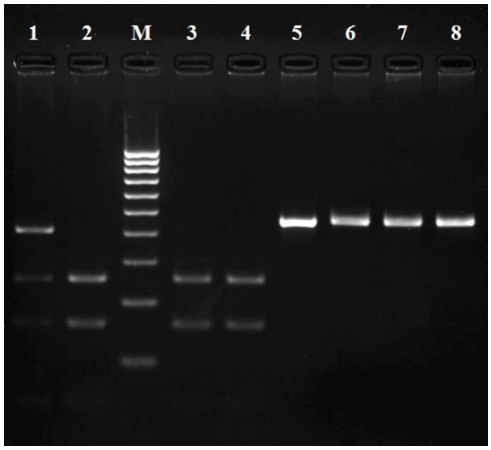


Figure. 3. PCR products of GDF9 gene and DNA electrophoretic pattern of amplicons after digestion with *AspLEI* restriction enzyme: lane M - 100 p.b. DNA ladder, lane 1 - CD genotype, lanes 2, 3, 4 - CC genotype, lane 5-8 - PCR products. Viewed on 3.0% agarose gel

The M allele of CAST gene and the homozygous MM genotype had the highest frequency in sheep of Tuvan breed (Table 1). Similarly the biggest value of frequency of C allele of GDF9 gene was in that population (Table 1). The largest part of population of sheep is homozygous of M and C alleles of CAST and GDF9 genes, respectively.

Table 1. The genotypes frequency for the CAST and GDF9 genes in Tuvan short-fat-tailed sheep breed in MUE "Chalaaty" (n=131)

| Genes | Genotypes | The number of animals | Genotypes frequency |
|---|---|---|---|
| CAST | MM | 112 | 0.850 |
| | MN | 19 | 0.145 |
| | NN | 0 | 0 |
| GDF 9 | CC | 115 | 0.878 |
| | CD | 16 | 0.122 |
| | DD | 0 | 0 |

In the examined group of sheep the most frequent was the M allele (0.928) and C allele (0.939), whereas the frequency of the N allele and the D allele was 0.072 and 0.061, respectively (Table 2).

Table 2. The alleles frequency for the CAST and GDF9 genes in Tuvan short-fat-tailed sheep breed from MUE "Chalaaty" (n=131)

| Genes | Alleles | Allele frequency |
|---|---|---|
| CAST | M | 0.928 |
| | N | 0.072 |
| GDF9 | C | 0.939 |
| | D | 0.061 |

Some researchers suppose this level of polymorphism is rather low and inconvenient for application in marker-assisted selection programs (Kolosov et al., 2015). But high total number of breed individuals can promote for search of sufficient number of animals with rare genotype for additional studying (Yuldashbaev et al., 2016).

## CONCLUSIONS

The genotyping of the population of Tuvan sheep breed for the CAST and GDF9 genes is one of the steps in the implementation of the candidate gene approach in sheep breeding. The allelic variants of the CAST and GDF9 gene were discovered in the process of that research**.** It can be useful to analyze the other groups of Tuvan short-fat-tailed breed from other agriculture organisations of the Tuva Republic to get more information of genotypes frequency. In the future additionally needs to investigate meat quality, weight traits and prolificacy of Tuvan Sheep for each genotype groups for ascertainment the fact of associating genotypes with level of those traits.

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