Identification of GDF5 gene polymorphism of bull-calves of the Kalmyk breed

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Abstract. The study covers bull-calves of the Kalmyk breed (n=182) aged 12-14 months. It considers the impact of polymorphism of the growth differentiation factor 5 (T586C in exon 1) ensuring development, maintenance and restoration of bones and cartilage on body parameters, body weight and musculoskeletal diseases. The frequency of occurrence of TT alleles in selection made 48.9%, TC – 46.7 and CC – 4.4%, χ2 test – 4.94. Bull-calves with CC genotype surpassed their analogues with TT and TC genotypes in terms of growth intensity and body measurements. The analysis of non-contagious musculoskeletal diseases (arthritis, bursitis, arthrobursitis) since birth until the 12th month of age revealed diseases among 17 heads (19.1%) with TT genotype, 9 heads (10.6%) with TC genotype and absence of diseases with CC genotype. There is a need for further study with increasing the group of animals with C homozygous genotype.

1. Introduction
The growth differentiation factor 5 (GDF5) is one of the genes participating in development, maintenance and restoration of bones and cartilages [1-3], known as the cartilage-derived morphogenetic protein-1 (CDMP1), which acts as the member of superfamily of the transforming growth factor-beta (TGF-b) and is closely connected with subfamily of bone morphogenetic proteins (BMPs) [4].

GDF5 acts as an extracellular signal molecule that activates the expression of genes participating in the formation of cartilages and bones [5], sinews and ligaments [6], playing a key role in cartilage recovery from injuries [7, 8].

The mutation of GDF5 gene may lead to serious musculoskeletal diseases that include joint dislocations mainly in knees and hips, shortening of extremity bones, phalanx joints disorders and brachydactyly [9-11]. Polymorphisms in GDF5 gene are connected with skeleton disorders, including various forms of chondrodysplasia, symphalangism [12, 13].

Considering the critical role of GDF5 gene in chondrogenesis and proteoglycan synthesis, which was proved on mice and humans [14-16], the study of the gene in relation to cattle may solve the problem of selecting animals resistant to musculoskeletal diseases, but it can also be used as a gene affecting growth intensity and body characteristics.
The purpose of the study was to analyze the influence of polymorphism in GDF5 gene on growth intensity, body measurements, incidence of musculoskeletal diseases of bull-calves of the Kalmyk breed.

2. Materials and methods
The protocol of the present study was approved by the Local Ethics Committee of Orenburg State University (Orenburg, Russia). All animal studies were performed in accordance with ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

2.1 Experimental design
The study was carried out in 2019 on bull-calves of the Kalmyk breed (n=182) aged 12-14 months. It considers the impact of polymorphism of the growth differentiation factor 5 (T586C in exon 1) ensuring development, maintenance and restoration of bones and cartilage on body parameters, body weight and musculoskeletal diseases.

The growth intensity of the studied animals since their birth to 12-month age was studied on the basis of individual monthly weight. The development was studied on the basis of the following measurements: height at the withers, height at hips, chest breadth, chest depth, body length, breadth at hips.

Non-contagious musculoskeletal diseases (arthritides, bursitides, arthrobursitides) were established on the basis of the registry log-book of sick animals.

2.2 Blood sampling and testing
DNA samples were taken from whole blood with using a set of reagents DIAtom™DNAPrep 200 (IsoGeneLab, Moscow). GenePak™PCRCore (IsoGeneLab, Moscow) and the EncycloPCRkit set (Evrogen, Moscow) were used for polymerase chain reaction. The primers were synthesized in R&D Company Litekh. Table 1 shows the nucleotide sequence of a primer for GDF5 marker gene.

| Gene-marker | Primer sequence                      | Product size, bps | Source           |
|-------------|--------------------------------------|-------------------|------------------|
| GDF5        | F: 5’-TGTCGATGCTGACAGAAAGG-3’        | 235               | Liu Y.F. et al.  |
|             | R: 5’-GAGTGAGGTTAATCCCAGATACCA-3’    |                   | (2010) [32]      |

PCR-RFLP of GDF5 gene was carried out within the MyCycler thermocycle (BioRad, USA). PCR protocol: initiating DNA denaturation within 5 minutes at 95°C, then 32 cycles of denaturation amplification at 94°C (30 sec.), annealing at 60°C (30 sec.) and elongation at 72°C (30 sec.), final synthesis at 72°C within 10 min.

The restriction reaction of received products of GDF5 amplification was carried out using Mval restriction endonuclease (Table 2).

| Gene | Restriction endonuclease | Base substitution | Incubation temperature, °C | Product size, bps |
|------|--------------------------|-------------------|----------------------------|-------------------|
| GDF5 | Mval                     | TC                | 37                         | TT – 235 bps      |
|      |                          |                   |                             | CC – 181 and 54 bps|
|      |                          |                   |                             | CT – 235, 181 and 54 bps|

For the reaction 20 mcl of PCR-product and 10 units of Mval were mixed in a test tube with subsequent incubation at t=37°C within 5 hours. The received product was divided by horizontal electrophoresis (in the 1st TBE at 80 V) in 2.5% agarose gel with coloring of ethydium bromide. Then gel was analyzed in ultra-violet light using UVTran-1 transilluminator, photography – VITran v.1.0 system.
The length of fragments was defined by the marker of molecular mass – GenePakR DNA Ladder M 50 (IsoGene Lab, Moscow).

2.3 Statistical processing
The Shapiro-Wilk test was used to check the hypothesis of normality of distribution of quantitative criteria. The distribution law of studied numerical indicators was not different from the normal one, therefore the significance of differences was checked by the Student’s t-test. All procedures of statistical analysis calculated the reached significance value (p), at the same time the critical significance value in this study was accepted smaller or equal 0.05. Statistica 10.0 application software package (Stat Soft Inc., USA) was used for data processing.

3. Results
SNP (T586C) in GDF5 gene showed three genotypes of “T>C mutation” (Fig. 1).

![Figure 1. Frequency of occurrence of genotypes according to GDF5 marker, heads](image)

The frequency of occurrence of TT alleles in selection made 48.9%, TC – 46.7 and CC – 4.4% ($\chi^2$ test is equal 4.94, with the frequency of alleles T = 0.72; C = 0.28).

The study of productive qualities of bull-calves of the Kalmyk breed regarding the growth intensity revealed considerable differences between the compared genotypes (Table 3).

| Age, months | Genotype | TT (n=89) | TC (n=85) | CC (n=8) |
|-------------|----------|-----------|-----------|----------|
| At birth    |          | 25.6±0.59 | 26.4±0.47 | 26.5±0.34 |
| 3           |          | 93.4±2.02 | 94.4±1.91 | 101.2±1.64ab |
| 6           |          | 162.3±2.41| 163.2±2.17| 177.2±2.02ab |
| 8           |          | 211.8±3.59| 214.1±3.48| 231.6±2.89ab |
| 12          |          | 314.7±4.17| 318.1±4.01| 339.4±3.84ab |

* P$\leq$0.05 – CC compared with TT;

b - P$\leq$0.01 – CC compared with TC.

The table shows average values (M) and arithmetic mean error (± m)
Bull-calves with CC genotype since the 3rd month showed high growth intensity (P≤0.05) in comparison with TT and TC genotypes.

The analysis of SNP marker association in GDF5 gene revealed its considerable influence on body measurements (Table 4).

**Table 4. Relation of SNP genotypes to body measurements, cm**

| Survey                  | Genotype |          |          |          |
|-------------------------|----------|----------|----------|----------|
|                         | TT (n=89)| TC (n=85)| CC (n=8) |          |
| Height at the withers   | 117.2±0.67| 117.8±0.82| 119.6±0.71 |          |
| Height at hips          | 120.1±0.93| 120.7±0.73| 122.4±0.67 |          |
| Chest breadth           | 38.4±0.34 | 38.9±0.32 | 39.6±0.27  |          |
| Chest depth             | 59.4±0.31 | 59.7±0.38 | 60.5±0.24  |          |
| Body length             | 140.2±0.91| 140.8±1.08| 143.6±0.76 |          |
| Breadth at hips         | 41.2±0.31 | 41.4±0.47 | 42.3±0.44  |          |

a - P≤0.05 – CC compared with TT;
b - P≤0.01 – CC compared with TC.
The table shows average values (M) and arithmetic mean error (± m)

Bull-calves with CC genotype were exceeding those with TT genotype in terms of breadth and height measurements, and with CT genotype only in terms of the body length.

The analysis of non-contagious musculoskeletal diseases (arthritis, bursitis, arthrobursitis) since birth until the 12th month of age revealed diseases among 17 heads (19.1%) with TT genotype, 9 heads (10.6%) with TC genotype and absence of diseases with CC genotype.

**4. Discussion**

Based on SNP definition [17, 18] DNA markers are considered as a key tool for genetic improvement of meat cattle heads. The selection of animals on the basis of genetic information allows forecasting the efficiency of received animals [19].

Polymorphism of many candidate genes is associated with indicators of cattle growth and efficiency, such as paired-like homeobox 1 (PROP1) [20], growth differentiation factor 10 (GDF10) [21], smoothened (SMO) [22], thyroglobulin (TG) [23], pleomorphic adenoma gene 1 (PLAG1) [24, 25], etc.

Limited study of the influence of GDF5 gene on productive qualities of cattle does not allow fully characterizing the gene and advisability of its use in selection of meat cattle. Impressive results are achieved in medicine highlighting the influence of the gene on growth [26, 27], skeletal muscles [28], development of musculoskeletal diseases [29-31], etc.

Based on genome-wide study of a person we applied the study of human GDF5 to the analysis of polymorphism and genetic impact on locus of GDF5 gene of cattle. This study shows that SNP (T586C) in GDF5 gene is connected with changes of cattle proportions and body weight. Gene mutations from TT to CC at their low frequency of occurrence (C = 0.28) was followed by the increase of body measurements: height at the withers, height at hips, chest breadth, chest depth, body length, breadth at hips (P≤0.05), which increased the body weight of calved when they reached 12 months by 7.8% (P≤0.001), which does not contradict earlier studies [32]. These data show that animals with TT genotype have reduced GDF5 expression in cartilage cells, which negatively affects the growth of bones thus reducing the overall growth of animals [33].

Besides, we revealed changes of non-contagious musculoskeletal diseases depending on gene polymorphism, but due to small selection in the group of homogeneous animals according to C allele, we cannot claim that the obtained data can be applied further. The study requires further detailed analysis and selection of animals with CC genotype taking into account the fact that the available study on
humans confirm the influence of GDF5 polymorphism in degenerative musculoskeletal diseases [34-37].

5. Conclusion
The conducted study highlights the influence of the genotype of bull-calves in terms of GDF5 gene on body measurements: height at the withers, height at hips, chest breadth, chest depth, body length, breadth at hips, which also changes the body weight of an animal.

The analysis of non-contagious musculoskeletal diseases revealed the influence of GDF5 gene polymorphism.

Further research increasing the group of animals regarding the homozygous C genotype.

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