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The polymorphism of *REM-1* gene in sheep genome and its influence on some parameters of meat productivity.

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ABSTRACT

One of new gene-candidate associated with meat productivity is REM-1 which can affect muscle development. REM-1 is a member of the RGK (Rem, Rem2, Rad, Gem/Kir) family of Ras-related monomeric GTP-binding proteins. We investigated the structure of regulatory regions and exons of the gene REM-1 and influence of polymorphisms on meat production in Russian sheep breed Dzhalginsky Merino. To detect alleles we use NimbleGen sequencing technology by Roche (USA). In this breed we found 14 single nucleotide polymorphisms (SNP) - three SNP in 3' and three in 5' flanking regions, four in exons and four - in 3'UTR. All of them are previously been entered into the dbSNP database and detected in some sheep breed's. Complex of six SNP c.*120, c.*193, c.*201, c.*332, c.*426, c.*474 and c.*623 are presented together in gene alleles and dont related with live weight and other parameters in heterozygous. SNP's c.-1417, c.-1415 and c.-1364 had positive effect on live weight, height, croup parameters and other in counted rams. Thus, the determination of allelic variants of REM-1 gene may be used in marker-assisted selection.

Keywords: REM-1, Sheep, Dzhalginsky Merino, SNP, Sequence

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INTRODUCTION

The implementation in practice of sheep marker-assisted selection methods based on the identification of the relationship between the structure of genes and animal productivity indicators. Therefore, the search for new candidate genes is primarily among those genes whose products regulate the growth and development of muscle fibers. One such gene is the REM-1 gene. On the base of the study about its expression in the muscle tissue of mice it was revealed the effect of this gene on the performance and dimensions of the body weight (Jerez-Timaure et al., 2005).

Rem-1 protein is a member of the RGK (Rem, Rem2, Rad, Gem/Kir) family of Ras-related monomeric GTP-binding proteins (Finlin and Andres, 1997). RGK proteins family affect the Ca^{2+} L-type channels (Béguin et al., 2001; Finlin et al., 2003; Yang et al., 2010). The general function of L-channels in the muscle is coupling of excitation and contraction (Bers, 2008). L-CaV1.1 channels in skeletal muscle function as a tension sensors (Melnikov, 2006). The ability of protein RGK to inhibit the work of CaV1.1 channels suggests that they may carry atrophic signaling. Thus, overexpression of the Rem genes has an adverse effect on the morphology of cultured myocytes (Romberg et al., 2014). In addition to the muscle fibers RGK proteins affect their vascular structures. The Rem-1 functions as a potent morphogenic switch in endothelial cells. Overexpression of its gene in cultured endothelial cells leads to the development of long cytoplasmic extensions and reorganization of the actin cytoskeleton. Experimentally it is shown that a mutation in codon 94 Rem-1 gene in humans with replacement of amino acids T to N leads to no endothelial cell sprouting (Pan et al., 2000). Based on the above data we can suggest the effect of the gene Rem-1 in the development of muscle tissue in farm animals. Researches of influence of gene polymorphisms REM-1 on the productive qualities of sheep have not conducted.

In sheep the Rem-1 gene is located on 13 chromosome, it has five exons. It is currently known about the three transcriptional variants (http://www.ncbi.nlm.nih.gov/gene. Accessed August 15, 2015). In the region of the gene Rem-1 was founded 162 single nucleotide substitutions (http://www.ncbi.nlm.nih.gov/snp. Accessed August 15, 2015).

In the Russian Federation, there are a number of sheep breeds that are bred by local breeders and adapted to life in the steppes territory of the North Caucasus. The "Dzhalginsky Merino" is a well-adapted breed of the dry conditions of the steppes of the Stavropol Krai with the distinguishing features of a combination of high wool and meat productivity (Dunin et al., 2013). The purpose of our researches were to investigate the structure of the Rem-1 gene in Dzhalginsky Merino for detecting of polymorphisms associated with high meat productivity.

MATERIALS AND METHODS

Sample collection

All work was done in the Genetic Laboratory of Science-Diagnostic and Veterinary Care Center (Stavropol State Agrarian University, Russian Federation). We investigated 15 Dzhalginsky Merino rams (n = 15) at an age of one year from a livestock breeding farm in Stavropol Krai, Russian Federation. In order to obtain data about the maximum number of *Rem-1* gene alleles we selected ten animals with maximum height and weight and five animals of the same population with a minimum height and weight. All animals were healthy, were kept in optimal conditions and were fed with a full ration.

DNA isolation

Genomic DNA was extracted from blood samples obtained from the jugular vein under aseptic conditions. Blood samples were collected in Vacutainer® vials with stabilizer EDTA (Becton Dickinson and Company, Franklin Lakes, NJ, USA) and were transported to the laboratory at +4 °C within 6 hours. DNA was extracted from 0.2 ml of blood using the PureLink Genomic DNA MiniKit (Invitrogen Life Technologies, Grand Island, NY, USA).

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Targeted enrichment and NextGeneration sequencing

In order to detect mutations in the genes, target enrichment was done and the investigated DNA fragments were sequenced. For enrichment of target regions, we used NimbleGen technology (Roche NimbleGen, Inc., Madison, WI, USA). Probes for target regions were developed in cooperation with Roche NimbleGen (USA). Libraries of DNA fragments from the investigated animals were prepared in accordance with the protocol in the Rapid Library Preparation Method Manual undergo the procedure of enrichment using NimbleGen SeqCap EZ Developer Libraries in accordance with the manufacturer protocol (Roche NimbleGen, Inc., Madison, WI, USA).

Monoclonal amplification of the enriched target regions of DNA was carried out according to a standard protocol in the emPCR Amplification Method Manual, Lib-L (Roche NimbleGen, Inc., Madison, WI, USA).

Sequencing was performed using a GS Junior genomic sequencer (Roche NimbleGen, Inc., Madison, WI, USA). The resulting sequences were mapped to the reference genome assembly Ovis aries oviAri3 (The National Center for Biotechnology Information. Genome. (2012) Ovis aries (sheep), 2015) by GS Reference Mapper v2.9 software (Roche NimbleGen, Inc., Madison, WI, USA).

To describe a single nucleotide polymorphism (SNP) we used HGVS nomenclature (www.hgvs.org). We used this nomenclature based on transcript XM_012188065.1 (https://www.ncbi.nlm.nih.gov/nuccore. Accessed 15 August 2015).

Statistical analysis

For statistical analysis, we used Student's t-test in Excel for Windows statistical plug-in. Significant difference detected if $p<0.05$.

RESULTS

Table 1: The frequency of *REM-1* polymorphic alleles and variants of genotype in Dzhalginsky Merino sheep breed

	Name of SNP in HGVS nomenclature	Positionincontig	Identifier in the NCBI database	Allele		Genotype		
1	c.-1417A>G	60377329	rs422672664	A	G	AA	AG	GG
				0.87	0.13	0.73	0.27	0.00
2	c.-1415T>G	60377331	rs399369974	T	G	TT	TG	GG
				0.87	0.13	0.73	0.27	0.00
3	c.-1364C>A	60377382	rs412664524	C	A	CC	CA	AA
				0.83	0.17	0.67	0.33	0.00
4	c.165T>C	60378910	rs160597902	T	C	TT	TC	CC
				0.87	0.13	0.73	0.27	0.00
5	c.447G>A	60384484	rs160597918	G	A	GG	GA	AA
				0.93	0.07	0.87	0.13	0.00
6	c.556A>G	60384593	rs429901478	A	G	AA	AG	GG
				0.40	0.60	0.07	0.66	0.27
7	c.888C>T	60386359	rs162014594	C	T	CC	CT	TT
				0.93	0.07	0.87	0.13	0.00
8	c.*120G>A	60386488	rs430025624	G	A	GG	GA	AA
				0.73	0.27	0.53	0.40	0.07
9	c.*193G>A	60386561	rs406345963	G	A	GG	GA	AA
				0.73	0.27	0.53	0.40	0.07
10	c.*201G>A	60386569	rs419654490	G	A	GG	GA	AA
				0.73	0.27	0.53	0.40	0.07
11	c.*332G>C	60386700	rs400256352	G	C	GG	GC	CC
				0.73	0.27	0.53	0.40	0.07
12	c.*426T>C	60386794	rs409737434	T	C	TT	TC	CC
				0.73	0.27	0.53	0.40	0.07
13	c.*474G>A	60386842	rs419067546	G	A	GG	GA	AA
				0.73	0.27	0.53	0.40	0.07
14	c.*623T>C	60386991	rs399441812	T	C	TT	5'C	CC
				0.73	0.27	0.53	0.40	0.07

We have found 14 single nucleotide substitutions: three SNP in the 3'and three in the 5' flanking regions, four in exons and four in the 3'UTR (Table 1). The predominant percentage mutation point's account for transitions - 79%, it is more often change purine bases. One of found SNP - c.556A>G results to the substitution in codon 186 of asparagine to aspartic acid.

The investigated animals according to identified mutations can be divided into five main groups and subgroups (total amount 10 genotypes). SNP c.*120G>A, c.*193G>A, c.*201G>A, c.*332G>C, c.*426T>C, c.*474G>A and c.*623T>C compile a group, and are found only in conjunction with each other.

The first combination of SNP represented with substitutions c.*120, c.*193, c.*201, c.*332, c.*426, c.*474 and c.*623. Since homozygous mutant variant has been in one animal, during evaluation the impact on the aggregate SNP on lifetime performance productivity its parameters were included in the group of replacements. The secondcombination is represented by the SNP c.-1417 and c.-1415, and found together only in four studied rams. The third SNP variant, whose influence on the productive qualities of the animals were examined, presented by one replacement - c.-1364. In 80% of cases, it occurs in combination with SNP c.-1417 and c.-1415.

Study of the effect on the production lifetime rates of the SNP c.*120-623 complex (Table 2) shows that its presence in the genome does not result in significant differences in any of measured parameters.

Table 2: Association between the *REM-1* genotypes and body measurements (n – number of animals; + - wild type allele; M – mutant allele; significantly differ with wild type homozygotes if p<0.05).

	Trait	Genotype		
		c.*120, c*193, c*201, c*332, c*426, c*474, c*623		
		+/+, M±m (n=8)	+/M, M±m (n=7)	p Value
1.	Liveweight (kg)	64.91±2.40	64.89±2.32	0.99
2.	Heightatwither (cm)	72.71±0.73	72.63±0.67	0.92
3.	Heightatcroup (cm)	75.71±0.51	74.50±0.67	0.15
4.	Widthatcroup (cm)	18.29±0.31	18.25±0.27	0.93
5.	Lengthofcroup (cm)	22.29±0.99	21.75±0.83	0.66
6.	Carcasslength (cm)	88.57±0.57	87.13±0.68	0.10
7.	Chestwidth (cm)	24.14±0.50	25.38±0.64	0.13
8.	Chestdepth (cm)	33.86±0.44	33.63±0.60	0.74
9.	Chest girth (cm)	97.71±2.57	97.50±1.48	0.94
10.	Metacarpalgirth (cm)	9.00±0.75	8.38±0.40	0.45
11.	Metacarpallength (cm)	16.14±0.44	16.13±0.24	0.97
12.	Metatarsuslength (cm)	17.43±0.40	17.38±0.28	0.91
13.	Loinwidth (cm)	15.29±0.31	15.13±0.32	0.70
14.	Widthofback (cm)	24.00±0.47	24.38±0.35	0.50
15.	Half girth of back (cm)	77.14±3.69	73.13±1.62	0.31

Animals with heterozygous genotypes in the SNP c.-1417 and c.-1415 were revealed significant differences with native wild homozygous genotype (Table 3). Live weight of wild homozygotes was 10% less than the heterozygotes. The same pattern has been found in a number of other parameters: height at wither was less in wild homozygotes by 3%, metacarpal length by 7%, metatarsus length by 6% and width of back - by 6%. In the analysis of other indicators of significant differences could not be detected.

Table 3: Association between the *REM-1* genotypes and body measurements (n – number of animals; + - wild type allele; M – mutant allele; significantly differ with wild type homozygotes if p<0.05).

	Trait	Genotype		
		c.-1417, c.-1415		
		+/+, M±m (n=11)	+/M, M±m (n=4)	p Value
1.	Liveweight (kg)	**63.03±1.75**	**70.05±1.58**	**0.01**
2.	Heightatwither (cm)	**72.00±0.51**	**74.00±0.47**	**0.01**
3.	Heightatcroup (cm)	74.82±0.56	75.75±0.55	0.22
4.	Widthatcroup (cm)	18.18±0.24	18.50±0.33	0.41
5.	Lengthofcroup (cm)	21.64±0.62	23.00±1.70	0.44

6.	Carcasslength (cm)	87.64±0.60	88.25±0.73	0.49
7.	Chestwidth (cm)	24.55±0.46	25.50±1.11	0.41
8.	Chestdepth (cm)	33.45±0.43	34.25±0.29	0.12
9.	Chest girth (cm)	95.82±1.15	102.50±3.04	0.08
10.	Metacarpalgirth (cm)	8.27±0.32	9.75±1.19	0.25
11.	Metacarpallength (cm)	**15.82±0.19**	**17.00±0.47**	**0.05**
12.	Metatarsuslength (cm)	**17.09±0.22**	**18.25±0.29**	**0.01**
13.	Loinwidth (cm)	15.18±0.24	15.25±0.55	0.90
14.	Widthofback (cm)	**23.82±0.24**	**25.25±0.55**	**0.05**
15.	Half girth of back (cm)	72.73±1.39	81.25±5.53	0.17

Most of the differences in lifetime rates of productivity has been found in animals that differ by the presence in the genome SNP c.-1364 (Table 4). Live weight of heterozygous individuals was more than 9% in compare with wild homozygotes. Height at wither was more than 2%, chest depth - 3%, metacarpal girth - by 27%, metacarpal length - 6%, metatarsus length - 7% and half girth of back - by 14%. The remaining parameters of productivity between the two groups have not differed significantly.

Table 4: Association between the *REM-1* genotypes and body measurements (n – number of animals; + - wild type allele; M – mutant allele; significantly differ with wild type homozygotes if p<0.05).

Trait		Genotype		
		c.-1364		
		+/+, M±m (n=10)	+/M, M±m (n=5)	p Value
1.	Liveweight (kg)	**62.99±1.94**	**68.72±1.90**	**0.04**
2.	Heightatwither (cm)	**72.10±0.60**	**73.80±0.42**	**0.03**
3.	Heightatcroup (cm)	74.70±0.61	75.80±0.42	0.13
4.	Widthatcroup (cm)	18.10±0.25	18.60±0.27	0.17
5.	Lengthofcroup (cm)	21.80±0.66	22.40±1.44	0.69
6.	Carcasslength (cm)	87.50±0.65	88.40±0.57	0.28
7.	Chestwidth (cm)	24.60±0.50	25.20±0.89	0.54
8.	Chestdepth (cm)	**33.40±0.50**	**34.40±0.27**	**0.09**
9.	Chest girth (cm)	96.10±1.23	100.60±3.11	0.19
10.	Metacarpalgirth (cm)	**8.30±0.35**	**10.60±0.57**	**0.01**
11.	Metacarpallength (cm)	**15.80±0.21**	**16.80±0.42**	**0.05**
12.	Metatarsuslength (cm)	**17.00±0.21**	**18.20±0.19**	**0.001**
13.	Loinwidth (cm)	15.10±0.23	15.40±0.45	0.54
14.	Widthofback (cm)	23.90±0.25	24.80±0.65	0.21
15.	Half girth of back (cm)	**72.50±1.52**	**82.60±3.82**	**0.04**

DISCUSSION

The nucleotide sequence of the *Rem-1* gene has a lot of variants even within the same breed of sheep. In Russia genotyping of sheep have not been conducted, there is no information about the structure of the genes in Russian breeds.

Rarely of all identified SNP found c.447G>A and c.888C>T, which was founded at the Moroccan sheep. Mutant alleles A and T in the genome of Moroccan sheep occur with equal frequency in 3% (http://www.ensembl.org/Ovis_aries/Gene/Variation_Gene. Accessed 15 August 2015). In our researches, the SNP is also rarely found, but the percentage of mutant alleles in Dzhalginsky Merino 2.3 times higher (7%), then that of Moroccan sheep.

The most common of found is a SNP c.556A>G. The mutant G allele occurs in 57% of Moroccan sheep and 75% of Iranian sheep. (http://www.ensembl.org/Ovis_aries/Gene/Variation_Gene. Accessed August 15, 2015). Dzhalginsky Merino sheep is characterized by the presence of the mutant allele G in 60% of cases, which is only 3% more than that of Moroccan sheep and 15% less than that of Iranian sheep.

The joint group of SNP c.*120-623, which we have found in Dzhalginsky Merino, we also found in some other breeds of sheep (http://www.ensembl.org/Ovis_aries/Gene/Variation_Gene. Accessed August 15, 2015). This confirms the assumption about joint inheritance of this group of substitutions. The mutant allele

c.*120-623 occur in Iran sheep in 43% cases. In case of Dzhalginsky Merino mutant alleles found in 1.5 times less, in 27% cases.

Moroccan sheep genome differs that SNP c.*120G>A is not linked with the group c.*193-623 and occurs both in conjunction with it, and independently (http://www.ensembl.org/Ovis_aries/Gene/Variation_Gene. Accessed August 15, 2015). However mutant allele c.*120A and mutant alleles in the group c.*193-623 occur with the same frequency 22%, which is 5% lower than in Dzhalginsky Merino and twice less than in Iranian sheep .

Our investigations about the link of individual SNP and their combinations on the lifetime productivity indicators of Dzhalginsky Merino sheep shown that these parameters are influenced by the substitutions, which are located in non-coding regions of the gene *REM-1*.

Found in Dzhalginsky Merino genome a complex of six SNP c.*120-623, located in the 3'UTR and downstream gene region in our investigations was not associated with live weight and other investigated productivity parameters. This may be due to the fact that out of four A-D genotypes, having in its composition all six SNP c.*120-623 half do not have a part of the gene of other found SNP – c.-1417, c.-1415 and c.-1364, whose effect on the size of the animal has been found. Maybe in a group of individuals with six substitutions in the gene structure the positive effect of SNP c.-1417, c.-1415 and c.-1364 in some individuals was compensated by genotypes without SNP in others. It was showed by the lack of reliable statistical difference between the native complex of six SNP and wild homozygotes.

In our study we have found the presence of a positive relationship in the genome SNP c.-1417 and c.-1415 with lifetime productivity parameters in Dzhalginsky Merino sheep. The greatest impact of heterozygous genotype was showed in relation to live weight, less - in relation to the parameters of the limbs bones size and width of back. The latter figure is determined by the development of muscle mass in the lumbar region and therefore related to the amount of meat taken from the carcasses.

The greatest value of the identified SNP in relation to the meat productivity has substitution c.-1364. The live weight parameter is affected by practically the same as the latest two substitutions. But the most contrast of wild homozygotes from heterozygotes for substitution c.-1364 was found in the measurement of the limbs bone bases. This indicates that the heterozygote has more robust constitution of the skeleton, which makes the animals hardier and more acceptable to muscle mass gain.

Thus, in choosing the SNP for marker-assisted selection in Dzhalginsky Merino sheep the most promising substitution is c.-1364. Its early identification in lambs will allow to select individuals looking for further fattening and breeding.

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