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Genetic variation of *RFXANK* gene in Stavropol sheep breed

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Marker assisted selection, a modern and promising method for accurate assessment and prediction of breeding and productive qualities of animals, allows fast breeding work at a reduced cost. For the development of genomic selection in sheep need to search for candidate genes associated with indicators of meat productivity.

For identification of single nucleotide polymorphism (SNP), 319 sheep were genotyped. One of the greatest impacts on the quality of the meat was substitution of s58995.1, located next to the gene *RFXANK* and possibly inherited clutch with him (Zhang *et al.* 2013). A similar mutation was also found in cows, rats and mice.

RFXANK gene is involved in regulation of gene expression of major histo-compatibility complex Class II (MHC II) (Masternak *et al.* 1998). Presence of the mutation site of the gene in humans causes severe combined immunodeficiency, accompanied by severe infectious diseases. In this case, the leucine at position 195 is replaced by proline, and the expression of MHC on the surface of the two B-cells is blocked (syndrome of "naked" lymphocyte, type 2) (Nekrep *et al.* 2001, Nagarajan *et al.* 2000). *RFXANK* gene expression was detected in thymus cells, lung and testes of the animals (Lin *et al.* 1999). High level of expression was detected in human skeletal muscle (Rader *et al.* 2000).

At the moment, NCBI database dbSNP contains information about 169 single-nucleotide substitutions in the gene *RFXANK* of sheep. There is no data about the frequency of occurrence of polymorphisms in different rocks at the moment.

Stavropol breed of sheep was created in the period from 1921 to 1950. The basis for the creation of the breed of fine-wool sheep were Novokavkazskie fine fleeced sheep. During the breeding, rams of American Rambouillet and Australian Merino were used. Among the wool breeds of sheep, Stavropol breed is the biggest in size and has good meat productivity, well adapted to the climate of Stavropol territory. Nowadays, Stavropol breed is one of the largest fine-wool sheep breeds in Russia (Aboneev *et al.* 2011).

The main objective was to study the structure of the *RFXANK* gene in sheep of Stavropol breed to identify polymorphisms associated with high meat productivity.

Healthy Stavropol rams (19), 1-year-old, were selected from the livestock breeding farm of Stavropol Krai. In order to obtain data about the maximum number of alleles, 13 rams with maximum growth and weight and 6 rams of the same population with a minimum height and weight, were selected. All animals were kept in optimal conditions.

Genomic DNA was extracted from blood samples obtained from the jugular vein under aseptic conditions. Blood samples were collected in vials with stabilizer EDTA and were transported to the laboratory at +4 °C within 6 h. DNA was extracted from 0.2 ml of blood using a kit.

In order to detect mutations in the genes, target enrichment and subsequent sequencing of the investigated DNA fragments were performed. For enrichment of target regions, we used the NimbleGen technology (Roche NimbleGen 2015). Probes for target regions were developed in cooperation with Roche NimbleGen (USA). Libraries of DNA fragments of investigated animals were prepared in accordance with the protocol Rapid Library Preparation Method Manual (Standard protocol GS Junior system manual, 2014) undergo the procedure of enrichment using NimbleGenSeqCap EZ Developer Libraries. Monoclonal amplification procedure of finished enriched target regions of DNA was carried out according to standard protocol emPCR Amplification Method (Standard protocol GS Junior system manual 2014).

Sequencing was performed using a genomic sequencer

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GS Junior. The resulting sequencing fragments mapped to the reference genome assembly Ovisaries oviAri3, August 2012 (NCBI Genome (2012) Ovisaries 2014) by software GS Reference Mapper v2.9.

In sheep, *RFXANK* gene is located on chromosome 5. In GenBank, there are 2 versions of the gene transcription: XM_004008404.1 and XM_004008405. They encode 2 different protein isoforms - XP_004008453.1 and XP_004008454.1 respectively. (NCBIOvisaries breed Texel chromosome 5, Oar_v3.1, whole genome shotgun sequence, 2014). In database of Ensembl project, there is another transcription: ENSOARG00000009235 (Ensembl project (2014) *RFXANK*, 2014).

To describe an SNP, we used HGVS nomenclature (The recommended nucleotide numbering nomenclature 2014).This nomenclature based on transcript 1-XM_004008404.1(NCBI).

The identified 20 SNPs are given in Table 1. All were previously included in the NCBI database. Predominant percentage of SNPs were transitions - 75%, with most change purine bases and mainly located in non-coding regions.

In the second exon, there is replacement of c.63C>A, which results to a change of leucine to phenylalanine at codon 21. In exon, 6 there was asynonymous substitution of c.498G> A. According to 20 detected SNPs investigated animals were divided into 4 main genotype.

In our opinion, indicators of meat productivity and the immune system can be affected by a mutation of c.63C>A

Table 1. The frequency of polymorphic alleles and variants of genotype in Stavropol sheep breed

	Name of SNP in HGVS nomenclature	Position in contig	Identifier in the NCBI database	Allele		Genotype		
1	c.-343	3830559	rs420126787	T	C	TT	TC	CC
				0.869	0.132	0.73	0.26	0
2	c.-111	3830327	rs408394637	T	G	TT	TG	GG
				0.869	0.132	0.73	0.26	0
3	c.63	3830154	rs160009883	C	A	CC	CA	AA
				0.895	0.105	0.79	0.21	0
4	c.187+62	3829968	rs428465729	G	A	GG	GA	AA
				0.789	0.211	0.58	0.42	0
5	c.187+328	3829702	rs410288357	G	A	GG	GA	AA
				0.869	0.132	0.73	0.26	0
6	c.187+394	3829636	rs427780973	C	T	CC	CT	TT
				0.632	0.368	0.32	0.63	0.05
7	c.187+469	3829561	rs416510636	A	G	AA	AG	GG
				0.105	0.895	0	0.21	0.79
8	c.187+623	3829407	rs426213487	G	A	GG	GA	AA
				0.763	0.237	0.58	0.37	0.05
9	c.187+711	3829319	rs415132827	G	C	GG	GC	CC
				0.447	0.553	0.16	0.58	0.26
10	c.188–670	3829228	rs425101219	A	G	AA	AG	GG
				0.237	0.763	0.05	0.37	0.58
11	c.188–588	3829146	rs398485199	G	C	GG	GC	CC
				0.789	0.211	0.58	0.42	0
12	c.188–127	3828685	rs411579647	C	T	CC	CT	TT
				0.789	0.211	0.58	0.42	0
13	c.338–94	3828125	rs418364857	G	A	GG	GA	AA
				0.869	0.132	0.73	0.26	0
14	c.498	3827694	rs405773959	G	A	GG	GA	AA
				0.869	0.132	0.73	0.26	0
15	c.565–44	3827318	rs422781984	A	G	AA	AG	GG
				0.895	0.105	0.79	0.21	0
16	c.632–124	3826975	rs420498050	G	A	GG	GA	AA
				0.869	0.132	0.73	0.26	0
17	c.712+309	3826462	rs422934569	C	T	CC	CT	TT
				0.658	0.342	0.32	0.68	0
18	c.712+354	3826417	rs404340119	C	T	CC	CT	TT
				0.895	0.105	0.79	0.21	0
19	c.713–107	3826063	rs424483467	G	T	GG	GT	TT
				0.632	0.368	0.32	0.63	0.05
20	c.713–82	3826038	rs413221323	G	A	GG	GA	AA
				0.869	0.132	0.73	0.26	0

in exon II, as it leads to the change of the peptide structure. The second exon includes part of 5'UTR and coding area (NCBI 2014). In the codon 21, leucine is replaced by phenylalanine. At the same time, it does not fall into one of the ankyrin repeats, which reduces the probability of expressed influence on the immune system. Carriers of this mutation in the heterozygous variant are 21% of the investigated animals, homozygous variant was not detected.

This is the first report of identifying SNPs in Russian sheep breed but all the SNPs were already reported in other breeds. The research indicates highly conserving of exon gene *RFXANK* and significant variability of noncoding regions. SNP, located in exons, can have a significant impact on the animals and meat productivity, parameters of the immune response. The remaining substitutions occurring in introns mainly in the form of combinations, in our opinion, may affect the splicing process and the formation of messenger RNA. On the basis of these results, it is necessary to continue to study and clarify the functional role of these mutations in the growth and development of muscles in sheep.

SUMMARY

Using of NimbleGen sequencing technology for detection of polymorphisms *RFXANK* gene in Stavropolsheep breed, we found 20 SNPs. That is, two SNP in exons - c.63C>A (non synonymous) in exon II and c.498G>A in exon VI (synonymous). Others SNP is in introns: c.-343T>C, c.-111T>G, c.187+328G>A, c.338-94G>A, c.632–124G>A, c.713-82G>A, c.187+62G>A, c.188-588G>C, c.188-127C>T, c.565-44A>G, c.712+309C>T, c.712+354C>T, c.712+309C>T, c.187+394C>T, c.187+469A>G, c.187+71G>C, c.188-670A>G, c.713-107G>T. Some of them are presented together.

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