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Myostatin gene (MSTN) polymorphism with a negative effect on meat productivity in Dzhalginsky Merino sheep breed

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Article info:
Received: 19 March 2015
Accepted: 6 July 2015

ABSTRACT

One of the most important negative regulator of muscle grow in mammalians is myostatin. Some mutations in myostatin gene (MSTN) can decrease the effect of protein and play role in meat quality of sheep. Therefore, in genome selection, knowledge of MSTN gene structure is very important. We investigated the polymorphism of the MSTN gene and its influence on body parameters in Russian sheep breed Dzhalginsky Merino. To detect alleles, we use NimbleGen sequencing technolog). In this breed, we found 20 single nucleotide polymorphism (SNP). That is SNP in promoter: c.-1866, c.-1404, c.-1401, c.-1213, c.-1128, c.-958, c.-783; 5'UTR: c.-40; exon I: c.101; intron 1-2: c.373+18, c.373+241, c.373+243, c.373+259, c.373+563; intron 2-3: c.747+164, c.747+309, c.748-810, c.748-229G>A, c.748-475; 3'UTR: c.*1232. Three of detected SNP (c.-1128, c.-958, c.-40) have a negative effect on the body parameters – decrease weight, height and other. Other three SNP (c.101, c.373+18, c.*1232) have not significant influence on this parameters. Our investigation is a base of next research of affection of different MSTN gene alleles on meat quality and can be used to prepare a PCR test-system for genomic selection.

Key words: Myostatin, MSTN, Sheep, Dzhalginsky Merino, SNP, Sequence

Introduction

Development of muscles and parameters of meat quality is controlled by large numbers of genes. One of the most important regulators of growth and development is the myostatin (MSTN). The protein encoded by this gene inhibits the development of muscle tissue of mammalians. It has been proven the link of some polymorphisms MSTN gene with increase of muscle mass in mice (McPherron et al., 1997), cattle (Grobet et al., 1997; Dunner et al., 2003), dogs (Mosher et al., 2007), pigs (Stinckens et al., 2008) and sheep (Boman & Våge, 2009; Han et al., 2010; Hickford et al., 2010).

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At the moment, the database NCBI (National Center for Biotechnology Information) includes information about more of 40 single-nucleotide substitutions in the myostatin gene of sheep (The National Center for Biotechnology Information. Database of Single Nucleotide Polymorphisms (2014) SNP at the Myostatin Gene (*Ovis aries*), 2015). Myostatin gene coding regions are highly conserved. It is known about two single nucleotide substitutions in exons. Missense mutation of c.101 G>A in the first exon leads to the substitution of glutamic acid for glycine. Substitution of c.384G>A in the second exon synonymous and does not change the encoded amino acid leucine (Zhou et al., 2008). Most mutations account for introns, 5'UTR and 3'UTR (The National Center for Biotechnology Information. Database of Single Nucleotide Polymorphisms (2013) Reference SNP (refSNP) Cluster Report: rs410961001, 2015).

Substitution of c.*1232 G>A in the 3'UTR of MSTN gene of sheep was offered to use as a marker for genomic selection (Kijas et al., 2007; Johnson et al., 2009; Masria et al., 2011; Han et al., 2013). According to the authors, the animals carrying two copies of the allele A have a broad chest and back, have a well-developed musculature. According to the results of VIA (video image analysis) in these animals significantly increased the estimated weight of the hind leg, chump and loin primal cuts (Masria et al., 2011).

In the North Caucasus (the southern region of the Russian Federation) has bred "Dzhalginsky Merino" breed, the distinguishing feature of which is the combination of high wool and meat productivity, well adaptation to the conditions of the dry steppes of the Stavropol Krai. The live weight of ram is 122.8 kg, dam - 55.6 kg, yearling rams - 79.5 kg, ewe - 41.3 kg, which is significantly higher than the standard requirements for sheep of wool breeds (Dunin et al., 2013).

In this context, the aim of our research was to study the structure of the MSTN gene of Dzhalginsky Merino sheep breed to identify polymorphisms, which can be associated with high meat productivity.

Materials and Methods

All work was provided in the Genetic Laboratory of Science-Diagnostic and Veterinary Care Center (Stavropol State Agrarian University, Russian Federation). We have investigated 30 rams (n=30) at the age of one year of Dzhalginsky Merino breed, from livestock breeding farm of Stavropol Krai, Russian Federation. In order to obtain data about the maximum number of myostatin gene alleles, we selected for the research 21 animals with maximum height and weight, and 9 animals of the same population with a minimum height and weight. All animals were healthy, kept in optimal conditions, and fed with a full ration.

DNA collection

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, USA) and were transported to the laboratory at +4°C within 6 hours. DNA was extracted from 0.2 ml of blood using a kit PureLinkGenomic DNA MiniKit (Invitrogen, USA).

Targeted enrichment and NextGeneration Sequencing

In order to detect mutations in the genes, we performed target enrichment and subsequent sequencing of the investigated DNA fragments. For enrichment of target regions we used the NimbleGen technology (Roche NimbleGen, 2015). Probes for target regions were developed in cooperation with the firm 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, 2015) undergo the procedure of enrichment using NimbleGen SeqCap EZ Developer Libraries (Roche NimbleGen, USA) in accordance with the protocol NimbleGen SeqCap EZ Library LRUser's GuideVersion 2.0 (2015).

Monoclonal amplification procedure of finished enriched target regions of DNA was carried out according to standard protocol emPCR Amplification Method Manual, Lib-L (Standard protocol GS Junior system manual, 2015).

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

To describe an SNP, we use HGVS nomenclature (The recommended nucleotide numbering nomenclature, 2015).

Statistical analysis

Phylogenetic analysis was performed using the software Unipro UGENE 1.15.1 (Unipro, Russia). For statistical analysis we used Student's t-test in Excel for Windows statistical plugin. Significant difference detected if $p<0.05$.

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Results and Discussion

During the sequencing, we have found 20 single nucleotide substitutions in myostatin gene (Table 1). According to our results, the predominant percentage of point mutations account for transitions - 70%, and often change pyrimidine bases. Detected SNPs were mainly located in non-coding regions. Only one change c.101A>G. is located in exon. In introns are located 10 substitutions, 7 substitutions in the promoter region, one in the 5'UTR and one in the 3'UTR (Table 2). Most often mutant alleles contained SNP c.-1128, c.-958, c.-40, c.373+18, c.373+259, c.747+164 and c.*1232. Their frequency in the investigated group was higher than 0.2, while c.-40 reached 0.4. The most rarely we met SNP c.-783, and only in the heterozygous form.

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

№	Name of SNP in HGVS nomenclature	Identifier in the NCBI database	Position in contig	Allele		Genotype		
1	c.-1866	rs418742295	118142577	C	T	CC	CT	TT
				0,875	0,125	0,8	0,15	0,05
2	c.-1404	rs412722044	118143039	A	T	AA	AT	TT
				0,9	0,1	0,8	0,2	0
3	c.-1401	rs424217443	118143042	G	A	GG	GA	AA
				0,875	0,125	0,75	0,25	0
4	c.-1213	rs398560354	118143230	C	T	CC	CT	TT
				0,875	0,125	0,75	0,25	0
5	c.-1128	rs414042681	118143315	T	C	TT	TC	CC
				0,685	0,315	0,47	0,33	0,2
6	c.-958	rs425338021	118143485	T	C	TT	TC	CC
				0,625	0,375	0,4	0,45	0,15
7	c.-783	rs403972675	118143660	G	A	GG	GA	AA
				0,95	0,05	0,9	0,1	0
8	c.-40	rs411139795	118144403	C	A	CC	CA	AA
				0,6	0,4	0,35	0,5	0,15
9	c.101	rs417816017	118144543	A	G	AA	AG	GG
				0,925	0,075	0,9	0,05	0,05
10	c.373+18	rs119102825	118144833	G	T	GG	GT	TT
				0,75	0,25	0,6	0,3	0,1
11	c.373+241	rs119102826	118145056	T	C	TT	TC	CC
				0,875	0,125	0,75	0,25	0
12	c.373+243	rs427811339	118145058	G	A	GG	GA	AA
				0,875	0,125	0,75	0,25	0
13	c.373+259	rs119102828	118145074	G	T	GG	GT	TT
				0,75	0,25	0,55	0,4	0,05
14	c.373+563	rs408710650	118145378	G	A	GG	GA	AA
				0,875	0,125	0,75	0,25	0
15	c.747+164	rs426500486	118147186	A	G	AA	AG	GG
				0,775	0,225	0,6	0,35	0,05
16	c.747+309	rs404916326	118147331	T	A	TT	TA	AA
				0,9	0,1	0,8	0,2	0
17	c.748-810	rs423466211	118148243	C	T	CC	CT	TT
				0,85	0,15	0,75	0,2	0,05
18	c.748-475	rs406265773	118148578	A	C	AA	AC	CC
				0,9	0,1	0,8	0,2	0
19	c.748-229	rs596160146	118148824	G	A	GG	GA	AA
				0,9	0,1	0,8	0,2	0
20	c.*1232	rs408469734	118150665	A	G	AA	AG	GG
				0,8	0,2	0,8	0	0,2

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Table 2. *Myostatin gene genotypes identified in Dzhalginsky Merino sheep breed.*

№	Genotype		*SNP*									
			Promoter							5'UTR	Exon I	Intron 1-2
			c.-1866	c.-1404	c.-1401	c.-1213	c.-1128	c.-958	c.-783	c.-40	c.101	c.373+ +18
1	A											
2	B	1										
3		2										
4	C											
5	D	1										
6		2										
7	E											
8	F	1										
9		2										
10		3										
11	G	1										
12		2										
13		3										
14		4										
	Genotype		*SNP*									
			Intron 1-2				Intron 2-3					3'UTR
			c.373+				c. 747+		c.748-			c.*1232
			+241	+243	+259	+563	+164	+309	-810	-475	-229	
15	A											
16	B	1										
17		2										
18	C											
19	D	1										
20		2										
21	E											
22	F	1										
23		2										
24		3										
25	G	1										
26		2										
27		3										
28		4										

Cell shaded in black indicate homozygous mutant allele, gray - heterozygous, white - homozygous wild-type allele.

Investigated animals have been divided in 7 main genotypes and subgroups according to 20 detected SNP (Table 2, Figure 1.). Group «A» includes 15% of the animals that no polymorphisms were found. The structure of the gene is identical to the Australian reference (Oar_v3.1). Samples, which have one change are included in the group "B" and are divided into two subgroups. Animals from "B1" are homozygous loci mutants c*1232, from "B2" - on c.101. The group includes 15% of the animals. Genotype "C" are small, represented in 5% of the samples. Animals in this group have 2 heterozygous SNP variant: c.-40C>A and c.373+18G>T. Animals from the group «D» are 20% of the total surveyed. They have 2 SNP in the promoter (c.-1128T>C, c.-958T>C)

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and one in the 5'UTR (c.-40C>A). Substitutions are shown in homozygous (D1) and heterozygous (D2) embodiments.

Genotype "E" has 5% of the investigated animals. They are characterized by the presence of 7 heterozygous substitutions: c.-1128T>C, c.-40C>A, c.373+241T>C, c.373+243G>A, c.373+259G>T, c.373+563G>A, c.747+164A>G.

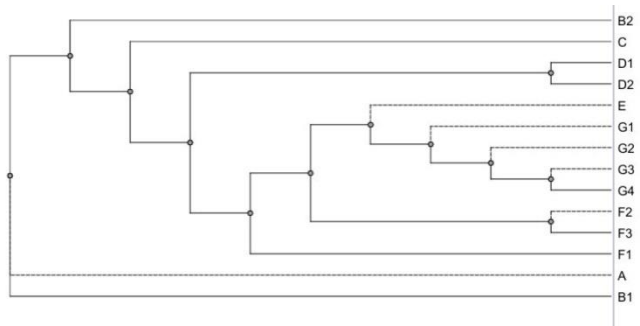

Figure 1. *Phylogenetic tree of detected genotypes.*

The largest number of substitutions had genotypes «F» and «G». They were found in 20% of cases. Animals from the group «F» have from 6 to 11 substitutions, from the group «G» - from 12 to 19.

The investigation of sheep genotypes variants depending on the combinations in SNP helped to identify three most common substitutions, in functionally important regions of the gene. These were replacements c.-1128, c.-958 and c.-40, two of which are located in the promoter and one in 5'UTR. These SNP in genotype are found in substantially together, only in variant of genotype E there is no substitution c.-958 and in the variant F1 - c.-1128. All three substitutions can occur both in the heterozygous and homozygous variants.

In this regard, we have investigated the relationship between the presence of these SNP, as well as substitutions c.*1232, c.-373+8, c.-101, and intravital indexes of sheep productivity. The investigation of intravital productivity index of sheep showed that there are significant differences in the animal's parameters that depend on the presence of specific alleles of gene MSTN (Table 3, Table 4).

Table 3. *Association between the MSTN genotypes and body measurements.*

	Trait	Genotype				
		c.-1128, c.-958, c.-40			c.*1232	
		+/+, M±m (n=14)	+/M, M±m (n=10)	M/M, M±m (n=6)	+/+, M±m (n=24)	M/M , M±m (n=6)
1.	Live weight (kg)	67.87±1.83	**61.55±1.89***	**56.87±1.31*#**	65.22±1.69	66.97±2.92
2.	Height at wither (cm)	73.14±0.28	72.63±0.70	**68.67±0.43*#**	72.92±0.47	71.67±1.63
3.	Height at croup (cm)	75.29±0.31	75.25±0.60	**72.67±1.08*#**	74.75±0.47	76.33±1.08
4.	Width at croup (cm)	18.43±0.22	18.13±0.32	18.06±0.41	18.17±0.22	18.67±0.41
5.	Length of croup (cm)	22.57±1.20	21.38±0.53	23.12±0.71	21.75±0.63	22.32±1.47
6.	Carcass length (cm)	88.43±0.46	87.38±0.81	86.03±1.43	87.42±0.52	**89.33±0.41***
7.	Chest width (cm)	24.71±0.52	24.63±0.70	24.33±1.47	24.92±0.42	24.33±0.78
8.	Chest depth (cm)	34.29±0.31	**32.88±0.43***	**32.31±0.41***	33.58±0.42	34.33±0.82
9.	Chest girth (cm)	97.71±2.37	96.50±1.51	94.01±1.21	98.33±1.60	**94.67±1.08***
10.	Metacarpal girth (cm)	9.02±0.71	8.38±0.45	9.32±1.08	8.75±0.48	8.33±0.41
11.	Metacarpal length (cm)	16.57±0.32	**15.75±0.27***	**15.11±0.73***	16.25±0.26	15.67±0.41
12.	Metatarsus length (cm)	17.86±0.28	17.29±0.39*	16.67±1.08	17.42±0.27	17.33±0.41
13.	Loin width (cm)	15.43±0.23	**14.88±0.30***	**14.67±0.44***	15.17±0.25	15.31±0.44
14.	Width of back (cm)	24.57±0.46	23.86±0.32	23.67±0.41	24.33±0.30	23.67±0.82
15.	Half girth of back (cm)	78.43±3.41	**71.88±1.53***	**69.67±1.08***	75.08±2.30	74.67±3.19

n – number of animals. Significantly differ with wild type homozygotes: * - $p<0.05$. Significantly differ with heterozygotes: # - $p<0.05$

The most marked differences in animals, which have single nucleotide substitutions c.-1128, c.-958 and c.-40 in the myostatin promoter, were found in the measurement of live weight. The live weight in individuals heterozygous for these mutations was significantly lower (by 8.2%), in comparison with the homozygous for the wild type. Even lower rates of live weight were found in carriers of the mutant allele in the homozygous variant. They had 7.6% lower live weight than in heterozygous and 16.3% lower than that of the wild type homozygotes.

Also, significant differences were found when measuring the height at wither and height at croup. These values were lower in the group of homozygous for the mutant type in comparison with heterozygotes and homozygotes for a wild

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allele. Height at wither was lower by 5.5% than heterozygous animals. Height at croup was also significantly lower in the mutant homozygotes by 3.4%, in comparison with heterozygotes. Animals with homozygotes wild genotype and heterozygous did not differ significantly from each other in these indexes.

Table 4. *Association between the MSTN genotypes and body measurements.*

	Trait	Genotype				
		c.-373+18			c.-101	
		+/+, M±m (n=18)	+/M, M±m (n=9)	M/M, M±m (n=3)	+/+, M±m (n=24)	M/M , M±m (n=6)
1.	Live weight (kg)	65.63±2.10	65.36±2.55	62.20±2.58	65.13±1.95	64.02±1.41
2.	Height at wither (cm)	73.03±0.59	72.80±0.55	72.67±0.82	72.50±0.54	73.32±1.08
3.	Height at croup (cm)	74.89±0.60	74.80±0.55	74.65±0.86	74.92±0.51	75.67±1.04
4.	Width at croup (cm)	18.33±0.25	18.01±0.35	17.69±0.44	18.17±0.22	18.62±0.48
5.	Length of croup (cm)	22.04±0.85	21.80±1.19	**20.62±0.47***	22.33±0.72	**20.63±0.40***
6.	Carcass length (cm)	87.67±0.56	87.60±1.04	87.34±1.49	87.75±0.58	88.04±0.72
7.	Chest width (cm)	25.31±0.53	24.40±0.57	24.35±1.11	24.67±0.43	25.31±1.64
8.	Chest depth (cm)	34.33±0.40	32.80±0.65	32.67±1.04	33.75±0.45	33.67±0.44
9.	Chest girth (cm)	98.11±1.65	97.20±3.17	96.39±1.72	97.75±1.54	97.24±3.77
10.	Metacarpal girth (cm)	8.67±0.47	8.60±0.97	8.09±0.14	8.83±0.48	8.09±0.24
11.	Metacarpal length (cm)	16.33±0.36	16.03±0.35	15.67±0.43	16.17±0.28	16.21±0.17
12.	Metatarsus length (cm)	17.67±0.31	17.11±0.37	**16.63±0.49***	17.31±0.27	17.67±0.49
13.	Loin width (cm)	15.22±0.29	15.20±0.42	**14.38±0.40***	15.33±0.23	14.61±0.42
14.	Width of back (cm)	24.56±0.36	23.80±0.42	**23.35±0.37***	24.17±0.34	24.33±0.48
15.	Half girth of back (cm)	75.78±2.10	74.60±4.78	**68.67±2.05***	75.92±2.22	71.33±2.86

n – number of animals. Significantly differ with wild type homozygotes: * - $p<0.05$. Significantly differ with heterozygotes: # - $p<0.05$

Chest depth in carriers of the homozygous genotype for the mutant allele was significantly lower by 5.8% than that of homozygotes for the wild allele. Also lower chest depth was observed in the heterozygote, but it was not significantly different in size from the homozygotes with mutated allele.

Metacarpal length was lower in homozygotes with mutant alleles and heterozygotes were not significantly differing among themselves and decreasing by 8.8% relative to the index in the wild allele homozygotes.

Loin width of homozygotes for the wild allele was significantly higher by 5.2% than in homozygotes for the mutant allele. At the same time indexes of heterozygotes and homozygotes mutant among themselves did not differ significantly.

Similar differences were found with respect to half girth of back. In mutant homozygotes index was significantly lower by 11.2% than homozygotes of the wild type. In homozygous of wild-type half girth of back did not differ significantly from heterozygotes.

Other parameters are listed in Table 3 were not significantly differed among themselves and were not depended on the presence of MSTN gene allele.

Analysis of the differences in intravital productivity of sheep depending on mutation of c*1232 showed that it affects significantly fewer parameters than previous substitutions. Carcass length of animals with mutation was significantly greater by 2.2%, than the homozygotes of wild type. But the magnitude of the change was very small.

On the chest girth presence of the mutation is affected as a significant reduction in the index by 3.8%, compared with homozygous for the wild type. On the other indexes we could not identify the influence of mutations.

During the investigation of the effect of substitution in the position of the c.-373+18 on indexes of intravital measurements it was found that homozygous for the wild allele and heterozygotes did not differ significantly between themselves. Only in homozygotes with a mutant allele of the gene MSTN was discovered a number of indexes with significant changes: length of croup, metatarsus length, loin width, width of back, half girth of back. However, their change is characterized by a few percent. Only the magnitude of half girth of back in homozygotes for the mutant allele was lower by 9.4% than homozygotes of wild-type.

In homozygous animals with the mutation c.-101, significant differences were found only in one index - length

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of croup, which would be less than in individuals with mutations at 7.7%. The other parameters of productivity between the two groups did not differ significantly.

MSTN gene has many variations. Genotypes differ greatly even within the same breed of sheep, not to mention the general population. In Russia, the genotyping of sheep have not been conducted and there is not information about the structure of the gene of Russian breeds.

In this research, we first study the structure of the gene MSTN of Dzhalginsky Merino breed of sheep. This breed is ideal for growing in the climatic conditions of Stavropol region, has high wool and meat productivity.

During the work we have identified 20 single nucleotide substitutions. These include the SNP in the promoter: c-1866, c-1404, c.-1401, c.-1213, c.-1128, c.-958, c.-783; 5'UTR c.-40; exon I: c.101; intron 1-2: c.373+18, c.373+241, c.373+243, c.373+259, c.373+563; intron 2-3: c.747+164, c.747+309 c.748-810, c.748-475; c.748-229 G>; 3'UTR: c.*1232.

According to reports the meat productivity is affected by 3 of 20 found nucleotide substitutions. Mutation of c.101A>G in the first exon alters the structure of the myostatin propeptide (Dunner et al., 2003). In the 34 codon glutamic acid is substituted by glycine. Since these two amino acids have big differences in the structure, it can be assumed that this change affects the ability of myostatin inhibition, and further on meat quality. This SNP found in Merino sheep, NC Romney, Corriedale and New Zealand hybrids (Han et al., 2010). Among carriers of New Zealand Romney mutations are 2% of the animals (Zhou et al., 2008). According to our studies, this mutation occurs in 10% of the animals homozygous embodiment only.

Mutation c.373+18G>T is located in intron 1-2, near the donor-site splice. It was shown at the Latvian dark-sheep (Sjakste et al., 2011), a number of New Zealand sheep breeds (Han et al., 2013), among representatives of Suffolk and Texel breeds (Kijas et al., 2007). A similar substitution (c.373+5G>A) is found in people with a double-muscle phenotype (Schuelke et al., 2004). Genetic changes in the border regions of introns may affect mRNA splicing, changing the amino acid sequence and, accordingly, meat quality (Sjakste et al., 2011). Among New Zealand Romney 28% of animals are homozygous on the c.373+18G>T, 26% of the animals are heterozygotes, 46% of sheep do not have mutation (Hickford et al., 2010). This replacement is quite common in Dzhalginsky Merino breed, although it is not as common as in Romney breed. Homozygous mutations have 5% of Dzhalginsky Merino rams, heterozygous - 35%; 60% of animals do not have mutation.

SNP c.*1232G>A is located in the 3'UTR. It is offered as a selection marker (Clop et al., 2006; Kijas et al., 2007; Johnson et al., 2009; Masria et al., 2011; Han et al., 2013). This mutation is common among texels: 75% of the animals are mutant with alleles AA, 16% - alleles AG, 9% of the alleles GG (Masria et al., 2011). Carriers are sheep of Lincoln, Poll Dorset, White Suffolk breeds (Kijas et al., 2007). In our research, we used a reference Oar_v3.1. Consequently adenine in position c.*1232 is the reference base, and guanine is a mutant. Accordingly, the mutation has the form c.*1232A> G. Investigated animals in 15% have mutant alleles in homozygous GG; 85% have wild-type alleles AA. Heterozygous for this substitution we have not identified.

In the database, we have not found information about the prevalence of certain mutations identified in Dzhalginsky Merino breed. These include c.-1866C>T, c.-1404A>T, c.-1401G>A, c.-1213C>T.

Mutations at loci c.-1128T>C and c.-958T>C are found in Dorset down breed, Merino breed, a number of New Zealand hybrids (Han et al., 2013). Substitution of c.-958T>C is spread the same way among Chinese breeds of sheep. The percentage of the wild type of allele T varies from 15 to 76%, the mutant allele C from 24 to 85% (Gan et al., 2008). According to our results, the prevalence of the T allele is 62%, C allele is 38%. There are both homozygous and heterozygous variants.

SNP carriers of c.-783G>A are sheep of a number of New Zealand and Chinese breeds (Gan et al., 2008; Han et al., 2013). According to our data substitution of c.-783G>A have 10% of rams of Dzhalginsky Merino breed.

Substitution of c.-40C>A is located in 5'UTR (Gan et al., 2008; Han et al., 2013; Kijas et al., 2007). The average percentage of mutant allele A for Merino, black and white Suffolk, Poll Dorset, Romney and a number of other breeds is 29% (Kijas et al., 2007). Among the investigated Dzhalginsky merino allele A is represented in 40%. Genotype CC has 35% of the animals, genotype CA has 50%, AA-genotype has 15%.

Substitutions of c.373+241T> C, c.373+243G>A, c.373+259G>T are located in intron 1-2 (Hickford et al., 2010; Sjakste et al., 2011; Farhadian et al., 2012; Han et al., 2013). The presence of these three SNP Iranian scientists has

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been allocated into separate genotype with a frequency of 41% (Farhadian et al., 2012). According to our results, the mutations of c.373+241T>C and c.373+243G>A have percentage of spreading to 25%. They are found only in the heterozygous form and in all cases, only in conjunction with each other. Most probably they are inherited linked. Substitution of c.373+259G>T has 45% of the investigated animals. GG genotype has 75% of rams, GT- 25%, TT-5%.

Mutation of c.373+563G>A previously has been found in New Zealand Guibriling (Han et al., 2013). Dzhalginsky Merino is a carrier of substitution in heterozygous variant in 25% of cases. Homozygotes with this SNP were not identified.

Substitutions of c.747+164A>G, c.748-810C>T described in sheep of Dorset Down, Poll Dorset Suffolk, New Zealand merino sheep and hybrid lines (Han et al., 2013). Among the investigated animals SNP in locus c.747+164 have 40% of rams, in locus c.748-810 have 25%.

Mutation of c.747+309T>A has been found by Chinese scientists (Gan et al., 2008). There are not prevalence data. According to our results, the TT genotype has 90% of the animals, and genotype TA has 10% of the animals.

Prevalence of substitutions c.748-475A>C and c.748-229G>A could not be found in the published data. Among the studied animals 20% of Dzhalginsky Merino are carriers of the heterozygous variant mutations c.748-475A>C. Mutant homozygotes were not found. Located in the second intron c.748-229G>A, carriers are 20% of the investigated animals. It is presented in the heterozygous form. In a population of wild-type alleles percentage is 90% G, mutant A - 10%.

Three SNP, whose influence on the size of the animal, we have found, are located in the 5' regulatory region of the myostatin gene. SNP c.-1128 is located approximately midway between the E-Box8 (CAAT) and Progesterone receptor binding site. Another substitute, c.-958, is located only in 12 nucleotides from the site of interaction with Myocyte enhancer factor 2 (MEF2) (Du et al., 2005). Thus, at least two of three substitutions can affect the functional properties of the gene promoter MSTN. And we have found SNP this effect may be of activating nature. At the same time production of myostatin may increase and be accompanied by reducing of animal's growth and weight, which was found during our investigation. Thus, the identification of these three mutations in sheep can tell us about their low productive value and can direct the selection of breeding towards consolidation of myostatinin allele the breed without the presence of the SNP.

Unfortunately, described for other breeds markers c.101G>A, c.373+18G>T and c.*1232G> A in this case were ineffective. As the results of the investigations, they have practically no influence on most of the parameters of intravital evaluating of sheep. In our opinion, this may be due to the fact that these substitutions are present in the genotypes with known negative SNP in the promoter. Thus, the negative effect of SNP is more negative and leads to a marked decrease of growth and weight gain of animals with positive markers for other breeds.

Conclusion

The study indicates highly conserved of exon gene MSTN and significant variability of noncoding regions. All of the SNP, found in sheep of Dzhalginsky Merino breed were discovered earlier and included in the NCBI database. We found three mutations, which have negative impact on some parameter of body in sheep. But three previously described like positive SNPs do not have a significant effect in our study. On the base of these results, we may correct breeding programs in order to further improve the breed by fixing Dzhalginsky Merino positive alleles in the population.

Acknowledgement

This project was funded by Ministry of Agriculture of the Russian Federation (agreement on the procedure and conditions for granting subsidies to financial security the state order for the provision of public services (works) by December 30, 2013 № 3119/13).

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