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Original Scientific Article

POLYMORPHISMS OF THE *IGF1* GENE IN RUSSIAN SHEEP BREEDS AND THEIR INFLUENCE ON SOME MEAT PRODUCTION PARAMETERS

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Summary: Insulin-like growth factor 1 (IGF-1) plays an important role in the growth and development of muscle tissue in animals. Research into the structure of *IGF1* in sheep may provide important information for genomic marker assisted selection used to increase meat production. We investigated the structure of the *IGF1* gene and the effect of polymorphisms on lifetime meat productivity performance in the Russian Soviet Merino sheep breed. Alleles were detected in 15 rams using NimbleGen sequencing technology by Roche (USA). 18 single nucleotide polymorphisms (SNPs) were found in this breed. Only one SNP – c.-81T>C – was found in the coding region. All other SNPs were located in introns 5'UTR and 3'UTR. The c.-5363C>T, c.-5188G>C, c.-5186G>A and c.-4088G>A polymorphisms, presented together in two alleles of the gene, correlate with a high live weight in a heterozygous state. The synonymous substitution of c.81T>C in the exon was not found to have any influence on the analyzed meat production parameters. One of the detected SNPs – c.-91A>C – had a positive correlation with weight, height, croup parameters and other attributes in rams.

Key words: sheep; gene; *IGF1*; meat; SNP; selection

Introduction

Genomic marker-assisted selection is now a major global sheep breeding trend. Therefore, the search for new genes whose structural features affect the productive qualities of the animals has become an urgent aim for the present stage of research.

The greatest numbers of known genes that affect sheep meat production code for various regulatory peptides such as myostatin (1), calpastatin (2) and others. Another important regulatory protein, which controls growth and development in mammalian muscle structures, is insulin-like growth factor 1 (*IGF-1*). Along with IGF-2, growth hormone (GH) and growth hormone releasing hormone (GHRH), it is a member of the so-called somatotropic axis (GH / IGF-1 axis), which plays a key role in the growth of vertebrates (3, 4). IGF-1 mediates the stimulatory effect of growth hormone and testosterone on the growth and development of muscle fibers (5, 6).

A number of genetic polymorphisms of the IGF1 gene were found to relate to growth parameters in chickens (12), pigs (13) and goats (4). Single

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nucleotide polymorphisms (SNPs) describe the impact in the *IGF1* gene on a number of productive performances of sheep (14, 15, 16, 17, 18). Thus, a wide range of physiological functions of IGF-1 can be attributed to the candidate genes to identify genetic markers of meat production in farm animals (19, 20, 21).

Correlations have been detected between the concentration of *IGF-1* in the plasma of various species, the size of their fetus and the live weight of newborns (4, 7, 8). A positive correlation was found between the level of IGF-1 in the plasma, birth weight and muscle eye depth (9, 10, 11) of lambs at the age of 100 days.

The Soviet Merino breed of sheep is one of the most common in the Russian Federation. This fine-wool breed of meat-wool sheep, bred in the Soviet Union during the 1930s, is well adapted to the dry steppe climate and grazing. It is characterized by a good exterior, strong constitution, well-proportioned physique, strong skeleton and correct statement of limbs. Sheep of this breed have higher than average meat production for a meat-wool combination (22).

To date, there are no investigations pertaining to the structure of *IGF1* gene from Russian breeds of sheep. With this in mind, the aim of the work was the discovery of polymorphisms in the *IGF1* gene in Soviet Merino breed of sheep and an assessment of their effect on meat production.

Material 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 rams (n = 15) at the age of one year of Soviet Merino breed, from livestock breeding farm of Stavropol Krai, Russian Federation. In order to obtain data about the maximum number of *IGF1* gene alleles we selected for the research 10 animals with maximum height and weight, and 5 animals of the same population with a minimum height and weight. All animals were healthy, were kept in optimal conditions and fed with a total mixed ration. To describe meat production analyzed parameters of body measurements.

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, 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 a kit PureLinkGenomic DNA MiniKit (Invitrogen Life Technologies, Grand Island, NY, USA).

Targeted enrichment and NextGeneration sequencing

In order to detect mutations in the genes there were performed target enrichment and sub-sequent sequencing of the investigated DNA fragments. For enrichment of target regions we used the NimbleGen technology (Roche NimbleGen, Inc., Madison, WI, USA). 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 undergo the procedure of enrichment using NimbleGen SeqCap EZ Developer Libraries in accordance with the protocol (Roche NimbleGen, Inc., Madison, WI, USA).

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

Sequencing was performed using a genomic sequencer GS Junior (Roche NimbleGen, Inc., Madison, WI, 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 NimbleGen, Inc., Madison, WI, USA).

To describe a single nucleotide polymorphism (SNP) we use HGVS nomenclature (www.hgvs.org). We used this nomenclature based on transcript XM_012159668.1 (The National Center for Biotechnology Information. Genome. (2012) Ovis aries (sheep), 2015).

Statistical analysis

Phylogenetic analysis was performed using the software Unipro UGENE 1.15.1 (Unipro, Russia).

For statistical analysis used Student's t-test in Excel for Windows statistical plugin. Significant diference detected if $p<0.05$.

Results

As a result of *IGF1* gene sequencing in the Soviet Merino sheep, we found 18 single nucleotide substitutions (Table 1). The percentage of point mutations accounting for transitions was 67%. The coding region of the gene consists of only one of the identified SNPs – c.81T> C. This substitution is synonymous and does not change the encoded amino acid.

Phylogenetic analysis has shown 13 variants of *IGF1* gene according to 18 detected SNPs. The investigated animals were divided into six main genotype groups (A-F). Groups C and D consisted of three subgroups; group F – of four subgroups. One genotype (A) is identical to the reference *IGF1* gene (OAR_v3.1) and was found in 13% of cases.

In order to investigate the influence on meat production, we selected SNPs with higher frequencies. Most detected substitutions occurred in heterozygous form; some were detected simultaneously in the same animal. According to the transcriptional variant XM_012159668.1, the jointly identified substitutions c.-5363C>T, c.-5188G>C, c.-5186G>A and c.-4088G>A are located in locus 5' of the regulatory region, 5'UTR and the first intron. The research on the effect of SNP presence on lifetime productivity indicators showed that body size in animals with a heterozygous genotype is not significantly different from body size in wild homozygotes (Table 2). Meanwhile, live weights in these two groups differed significantly – by more than 4.5 kilograms.

The only substitution found in the exon (substitution c.-81T>C) had an insignificant effect on the lifetime productivity evaluation of any heterozygous or homozygous variant of Soviet Merino sheep.

The research on the impact of the c.-91A>C substitution on the body and live weight of the animals (half of which had the substitution) yielded interesting results (Table 2).

The carrier of homozygous mutant genotype of substitution c.-91A>C was only one of the investigated rams (shown in the last column of table 2). As shown in the table, the weight parameters are in the range of the group with the wild homozygous genotype. However, body size varies significantly in comparison with animals from the heterozygous group. A comparison between animals with wild homozygous and heterozygous genotypes revealed significant differences on a number of indicators: the average live weight of the heterozygotes was significantly greater than that of the homozygotes by 5.7%. The wither height was 4.2% greater in the heterozygotes, while the difference in croup height was 3.5%. The croup width of the heterozygous sheep was higher than the homozygotes by an average of 8.9%, while the croup length of the heterozygotes was a significant 6.6% greater. In addition, the back width was more than 4.4% the value of wild homozygotes. The dimension of back girth was greater in rams with the heterozygous genotype by 4.7%.

Discussion

Our studies of the individual link SNPs and their combinations on the lifetime productivity indices of the Soviet Merino sheep breed have shown that substitutions located in noncoding areas have an impact on a number of parameters. At the same time, the presence of the SNP c.-81 genotype located in the exon area, previously described as a g.271C>T (18) and recommended as a genetic marker (16), does not affect the animals' body size. This may be due to the fact that the substitution is synonymous and not accompanied by a change in the amino acid sequence of the protein produced. Due to the fact that, in a sample with different quantities of live weight (and only in the heterozygous form), the substitution is common in three-quarters of the animals, it cannot be used as a productivity marker.

In our opinion, a complex of SNP c.-5363C>T, c.-5188G>C, c.-5186G>A and c.-4088G>A, could be considered as markers of increased meat production in the investigated sheep breeds. There are several reasons for this. Firstly, it is found in 2.5 times fewer cases than the wild homozygous genotypes; secondly, it revealed a significant correlation with the live weight of animals, which

Table 1: The frequency of *IGF1* gene polymorphic alleles in Soviet Merino sheep breed

	Name of SNP in HGVS nomenclature	RefSNP(rs) number	Genomic location	Biotype of SNP	Allele		Genotype		
1	c.-5412A>G	rs423903256	171328624	Upstream gene variant	T	C	TT	TC	CC
					0.83	0.17	0.8	0.07	0.13
2	c.-5363C>T	rs412597723	171328575	Upstream gene variant	G	A	GG	GA	AA
					0.9	0.1	0.8	0.2	0.00
3	c.-5188G>C	rs401028781	171328400	5'UTR variant	C	G	CC	CG	GG
					0.87	0.13	0.73	0.27	0.00
4	c.-5186G>A	rs422604851	171328398	5'UTR variant	C	T	CC	CT	TT
					0.87	0.13	0.73	0.27	0.00
5	c.-4088G>A	rs400113576	171327300	Intron variant	C	T	CC	CT	TT
					0.9	0.1	0.8	0.2	0.00
6	c.-4032G>A	rs421570650	171327244	Intron variant	C	T	CC	CT	TT
					0.97	0.03	0.93	0.07	0.00
7	c.-91A>C	rs430457475	171323303	Intron variant	T	G	TT	TG	GG
					0.7	0.30	0.47	0.47	0.06
8	c.81T>C	rs159876393	171323132	Synonymous variant	A	G	AA	AG	GG
					0.5	0.5	0.20	0.60	0.20
9	c.151+199G>A	rs402729264	171322863	Intron variant	C	T	CC	CT	TT
					0.7	0.3	0.40	0.60	0.00
10	c.151+463A>C	rs422974179	171322599	Intron variant	T	G	TT	TG	GG
					0.93	0.07	0.87	0.13	0.00
11	c.152-159G>A	rs424410885	171270326	Intron variant	C	T	CC	CT	TT
					0.80	0.20	0.67	0.27	0.06
12	c.152-47C>A	rs413216906	171270214	Intron variant	G	T	GG	GT	TT
					0.80	0.20	0.67	0.27	0.06
13	c.333+7C>T	rs419007446	171269979	Intron variant	G	A	GG	GA	AA
					0.83	0.17	0.73	0.20	0.07
14	c.333+88T>C	rs400681017	171269898	Intron variant	A	G	AA	AG	GG
					0.83	0.17	0.73	0.20	0.07
15	c.333+164C>T	rs406373781	171269822	Intron variant	G	A	GG	GA	AA
					0.87	0.13	0.80	0.13	0.07
16	c.333+259T>A	rs427977916	171269727	Intron variant	A	T	AA	AT	TT
					0.87	0.13	0.73	0.27	0.00
17	c.333+435A>G	rs420696231	171269551	Intron variant	T	C	TT	TC	CC
					0.83	0.17	0.73	0.20	0.07
18	c.*72C>G	rs159876382	171254333	UTR variant 3 prime	G	C	GG	GC	CC
					0.97	0.03	0.93	0.07	0.00

Table 2: Body measurements of rams with different *IGF1* genotypes (n represents number of animals; + represents wild type allele; M represents mutant allele; significantly different from wild type homozygotes if p<0.05)

Trait	Genotype						
	c.-5363, c.-5188, c.-5186, c.-4088			c.-91			
	+/+, (M±m, n=11)	+/M, (M±m, n=4)	p value	+/+, (M±m, n=7)	+/M, (M±m, n=7)	P value	M/M, (n=1)
Live weight (kg)	**51,18±0,96**	**55,83±0,27**	**0,001**	**49,70±0,98**	**52,51±0,23**	**0,04**	50,3
Height at wither (cm)	70,67±0,96	71,33±0,82	0,57	**69,50±1,07**	**72,43±0,51**	**0,02**	73
Height at croup (cm)	73,25±0,83	72,33±1,47	0,56	**71,88±0,87**	**74,41±0,54**	**0,02**	75
Width at croup (cm)	16,92±0,42	17,67±1,08	0,50	**16,13±0,32**	**17,57±0,47**	**0,03**	17
Length of croup (cm)	21,00±0,36	20,67±0,41	0,51	**20,12±0,30**	**21,45±0,52**	**0,04**	21
Carcass length (cm)	79,33±1,26	81,00±0,71	0,23	79,25±1,24	79,57±1,63	0,87	76
Chest width (cm)	23,83±0,46	24,33±1,47	0,73	24,13±0,71	23,71±0,51	0,62	24
Chest depth (cm)	30,42±0,58	31,00±1,22	0,64	30,88±0,79	30,14±0,64	0,46	31
Chest girth (cm)	99,08±1,12	99,00±2,83	0,98	98,63±1,56	99,57±0,74	0,57	99
Metacarpal girth (cm)	10,67±0,23	12,67±2,68	0,46	10,75±0,34	11,44±1,02	0,52	11
Metacarpal length (cm)	17,08±0,33	16,33±0,82	0,38	16,71±0,44	17,14±0,44	0,51	18
Metatarsus length (cm)	18,42±0,33	18,00±0,71	0,57	18,38±0,40	18,29±0,45	0,88	19
Loin width (cm)	13,42±0,24	14,00±0,71	0,42	13,36±0,35	13,71±0,31	0,45	13
Width of back (cm)	24,17±0,36	23,33±2,04	0,67	**23,71±0,39**	**24,75±0,27**	**0,03**	25
Half girth of back (cm)	78,33±1,25	73,33±7,12	0,48	**76,86±1,40**	**80,50±0,93**	**0,04**	83

represents an integrative measure of productive qualities; thirdly, the substitutions in positions c.-5188, c.-5186 (previously described in the article as g.855G>C), g.857G>A (16), C1511G, A1513G (17), g.179T>C and g.181G>C (23) have been proposed as genetic markers. In this regard, it is desirable that the directed mating of heterozygous animals with these substitutions be conducted in order to produce homozygous mutant allele carriers, which may have more pronounced upward deviations in weight. The absence of changes in the external measurement of the investigated animals with heterozygous genotypes does not form a valid basis for conclusions concerning the low markers of the investigated SNP complexes. There is a strong possibility that a more detailed investigation of such indicators in slaughtered animals could reveal a significant shift in the ratio of muscle mass, bone basis weight and internal organs.

The most important factor in the evaluation of the productive qualities of Soviet Merino breed of sheep is *IGF1* gene polymorphism at position c.-91. Heterozygous individuals that make up half of the samples are characterized not only by higher live weights, but also by an increase in the external body dimensions. Moreover, an increase was observed in parameters such as height, croup and back size, the last two of which are entirely dependent on the development of the muscles of the animals. From

this, it can be concluded that when a heterozygous variant of SNP c.-91A>C is detected in a lamb, it is possible to predict its parameters of superior meat production with a high degree of certainty. An examination of a single individual in a sample with homozygous mutant genotypes indicates that the use of this marker for breeding of animal lines with similar genotypes will eliminate wild homozygotes and increase productive performance across the whole breed.

Conclusion

The study indicates high variability of noncoding regions of the *IGF1* gene in sheep. A complex of four SNP c.-5363C>T, c.-5188G>C, c.-5186G>A and c.-4088G>A are presented together in two alleles, correlating with increasing live weight in heterozygotes. Substitution c.81T>C in exons is synonymous and did not affect any parameters of meat productivity. SNP c.-91A>C had a positive effect on weight, height, croup parameters and other variables in counted rams. New markers for marker-assisted selection in *IGF1* gene alleles were found.

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POLIMORFIZMI GENA *IGF1* PRI RUSKIH PASMAH OVC IN NJIHOV VPLIV NA NEKATERE PROIZVODNE PARAMETRE MESA

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Povzetek: Inzulinu podoben rastni faktor 1 (*IGF-1*) ima pomembno vlogo pri rasti in razvoju mišičnega tkiva pri živalih. Raziskave strukture gena IGF1 pri ovcah lahko zagotovijo pomembne nove podatke za selekcijo za povečanje prireje mesa na osnovi genetskih označevalcev. Proučevali smo strukturo gena *IGF1* in vpliv polimorfizmov na prirejo mesa pri ovcah rusko-sovjetske merino pasme. S tehnologijo NimbleGen za določanje zaporedja baznih parov smo analizirali alaele pri 15 ovnih. Našli smo 18 mononukleotidnih polimorfizmov (SNP), od katerih je bil samo en SNP - c-81T> C v kodirajočem področju. Vsi drugi SNP-ji so bili v intronih, 5'-UTR in 3'-UTR. Polimorfizmi c.-5363C> T, c-5188G> C, c-5186G> A in c.-4088G> A, ki so skupaj v dveh alelih, so bili v sorazmerju z višjo živo težo živali, če so bile te heterozigotne za ta alel. Sinonimna zamenjava c.81T> C v eksonu gena ni vplivala na analizirane parametre prireje mesa. Eden izmed odkritih SNP-jev – c.-91A> C – je bil pozitivno povezan s težo, višino, parametri v križu in drugimi latnostmi ovnov.

Ključne besede: ovce; gen *IGF1*; meso; SNP; izbor