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SEQUENCING OF CANDIDATE GENES ASSOCIATED WITH VALUABLE AGRICULTURAL TRAITS IN SHEEP OF KAZAKH SELECTION

This study concentrates on the sequencing of candidate genes linked to desirable agricultural qualities in Kazakh selection sheep, a region of considerable significance in improving sheep breeding and agricultural practices. The main goal is to examine genetic markers that impact important characteristics like as meat and wool quality, which are vital for the financial sustainability of sheep farming. The study focuses on certain candidate genes, namely MSTN, CAST, FAT1, and FZD3, which have been identified for their potential influence on agricultural features, using modern genetic sequencing techniques. The process entails the sequencing of specific sections of the genome and conducting a thorough examination of these genes to detect changes and their associations with desirable agricultural characteristics. The findings unveil significant genetic differences that are directly linked to the qualities of interest. These findings are crucial not only for comprehending the genetic composition of Kazakh sheep but also for their substantial impact on the domain of animal genetics and breeding. This work significantly enhances the current understanding by clarifying the genetic elements that impact important agricultural characteristics in sheep. Moreover, the discovery holds practical importance due to its potential applicability in selective breeding schemes. Through utilizing the knowledge acquired from genetic analysis, breeders can devise more effective and focused breeding tactics, ultimately resulting in enhanced agricultural output and quality in sheep farming. Consequently, this research shows great potential for progressing the field of animal genetics and enhancing the economic viability of sheep farming in Kazakhstan and other regions.

Key words: sequencing, candidate genes, valuable traits, sheep.

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Қазақ қой саласында бағалы ауылшаруашылық сипаттарымен байланысты кандидат гендерінің тізілімі

Бұл зерттеу қой шаруашылығы мен ауылшаруашылық тәжірибесін жақсарту үшін маңызды аймақ болып табылатын қазақ тұқымды қойлардағы қалаулы ауылшаруашылық белгілерімен байланысты кандидат гендерін секвенирлеуге бағытталған. Негізгі мақсат – қой шаруашылығының қаржылық тұрақтылығы үшін маңызды ет пен жүн сапасы сияқты маңызды белгілерге әсер ететін генетикалық маркерлерді зерттеу. Зерттеу қазіргі заманғы генетикалық секвенирлеу әдістерін қолдана отырып, ауылшаруашылық белгілеріне әлеуетті әсерлері үшін анықталған MSTN, CAST, FAT1 және FZD3 сияқты нақты кандидат гендерге назар аударды. Бұл процесс геномның белгілі бір аймақтарын секвенирлеуді және өзгерістерді және олардың қажетті ауылшаруашылық сипаттамаларына қатынасын анықтау үшін осы гендердің егжей-тегжейлі зерттеулерін жүргізуді қамтиды. Нәтижелер қызығушылық белгілеріне тікелей байланысты маңызды генетикалық айырмашылықтарды көрсетеді. Бұл нәтижелер қазақ қойларының генетикалық құрамын түсіну үшін ғана емес, сонымен қатар олардың генетика мен мал шаруашылығы саласына айтарлықтай

әсер етуімен де маңызды. Бұл жұмыс қойдың маңызды ауылшаруашылық сипаттамаларына әсер ететін генетикалық элементтерді нақтылау арқылы қазіргі түсінікті айтарлықтай кеңейтеді. Сонымен қатар, ашылу оның асыл тұқымды схемаларда әлеуетті қолданылуына байланысты практикалық әсерге ие. Генетикалық талдаудан алынған білімді пайдалана отырып, селекционерлер анағұрлым тиімді және мақсатты асылдандыру тактикасын жасай алады, бұл сайып келгенде, ауыл шаруашылығы өнімі мен қой өнімінің сапасын арттыруға әкеледі. Сондықтан бұл зерттеу жануарлар генетикасы саласын ілгерілету және Қазақстандағы және басқа аймақтардағы қой шаруашылығының экономикалық тиімділігін арттырудың үлкен әлеуетін көрсетеді.

Түйін сөздер: секвенирлеу, кандидат гендер, құнды белгілер, қойлар.

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Секвенирование генов-кандидатов, ассоциированных с ценными сельскохозяйственными признаками у овец казахской селекции

Настоящее исследование сосредоточено на секвенировании генов-кандидатов, связанных с желаемыми сельскохозяйственными качествами у овец казахской селекции, региона, имеющего большое значение для улучшения овцеводства и методов ведения сельского хозяйства. Основная цель — изучить генетические маркеры, которые влияют на важные характеристики, такие как качество мяса и шерсти, которые имеют жизненно важное значение для финансовой устойчивости овцеводства. Исследование сосредоточено на определенных генах-кандидатах, а именно MSTN, CAST, FAT1 и FZD3, которые были идентифицированы по их потенциальному влиянию на сельскохозяйственные характеристики с использованием современных методов генетического секвенирования. Этот процесс включает в себя секвенирование определенных участков генома и проведение тщательного изучения этих генов для выявления изменений и их связи с желаемыми сельскохозяйственными характеристиками. Результаты раскрывают значительные генетические различия, которые напрямую связаны с интересующими качествами. Эти результаты имеют решающее значение не только для понимания генетического состава казахских овец, но и из-за их существенного влияния на область генетики и селекции животных. Эта работа значительно расширяет нынешнее понимание, уточняя генетические элементы, которые влияют на важные сельскохозяйственные характеристики овец. Более того, открытие имеет практическое значение из-за его потенциального применения в схемах селекции. Используя знания, полученные в результате генетического анализа, селекционеры могут разработать более эффективную и целенаправленную тактику разведения, что в конечном итоге приведет к увеличению сельскохозяйственной продукции и качества овцеводства. Следовательно, это исследование показывает большой потенциал для развития области генетики животных и повышения экономической рентабельности овцеводства в Казахстане и других регионах.

Ключевые слова: секвенирование, гены-кандидаты, ценные признаки, овцы.

Introduction

Sheep farming is one of the most important areas of animal husbandry, as sheep are valuable sources of meat, wool, leather, and milk. In recent years, researchers around the world have been actively studying the genetic characteristics of sheep to improve their agricultural characteristics, such as disease resistance, wool quality, body weight, and meat yield [1, 2].

One approach to identifying genetic markers associated with desirable agricultural traits in sheep is to use candidate genes. Candidate genes are genes that are thought to play a role in the development of

certain traits based on their function or location in the genome [3-8].

In this article, we investigated candidate genes associated with valuable agricultural traits in sheep and their connection to these traits. The use of genetic markers in sheep breeding is an important tool for improving the agricultural characteristics of the herd. They can help increase productivity, endurance, adaptation to various environmental conditions, improve product quality, and reduce herd maintenance costs. As part of the study, we analyzed the genes MSTN, CAST, FAT1, and FZD3, which are known for their influence on muscle growth and development, wool quality, and adipose tissue in sheep.

The MSTN gene, also known as the myostatin gene, is a genome that plays an important role in regulating muscle growth and development. Specifically, the MSTN gene produces a protein called myostatin, which is a negative regulator of muscle mass. Mutations in the MSTN gene in sheep can lead to increased muscle mass and improved meat quality, making it an important target for cattle breeding programs [9-12].

The CAST gene is pivotal in encoding calpastatin, a protein calpastatin, which is a specific inhibitor of the protease calpain. Calpastatin plays an important role in regulating the activity of calpain in cells, which is important for a wide range of physiological processes, including muscle growth, protein development and metabolism. Studies have shown that changes in the CAST gene can affect the tenderness of meat, which is a highly desirable property in the meat industry [13].

The gene FAT1 in sheep encodes the protein FAT1, which is a member of the cadherin family of cell adhesion molecules. FAT1 is involved in several cellular processes, including cell polarity, migration, and tissue development [14-16].

The gene FZD3 in sheep encodes the protein Frizzled-3, which is a receptor for the Wnt signaling pathway. The Wnt signaling pathway plays an important role in regulating various developmental processes, including cell proliferation, differentiation, and tissue morphogenesis. Variations in the FZD3 gene are associated with differences in wool fiber diameter, fiber strength, and frequency of waves in sheep wool [17, 18].

To summarize, the continuous investigation into genetic markers for sheep breeding is of utmost significance. The technology has the capacity to revolutionize sheep farming by increasing productivity, enhancing disease resistance, facilitating adaptation to environmental changes,

and promoting sustainable and ethical breeding techniques. This research is essential for satisfying the increasing worldwide need for sheep products and also serves a critical function in conserving genetic variety and enhancing the economic sustainability of sheep farming. Therefore, it signifies a crucial domain of concentration in the field of agricultural genetics and animal management.

Materials and Methods

In order to determine the nucleotide sequence of the genome and the associated agricultural traits, partial genome sequencing was performed on an ABI PRISM 310 instrument (Applied Biosystems) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) reagent set. Prior to sequencing, target DNA was amplified using specific primers, the information of which is presented in Table 1. The following components were required for the amplification reaction: 300 ng of target DNA, 3.2 pM of primer, 0.75X sequencing buffer, 0.25X terminator, and volume adjustment to 10 µl with deionized water. A program compatible with the ABI PRISM 310 genetic analyzer protocol was used for sequencing.

The amplification program consisted of the following steps: 1. Initial denaturation, 5 min – 94°C; 2. Cycling. 35 cycles: a) denaturation, 1 min – 94°C; b) primer annealing, 1 min – 62°C; c) elongation, 1 min – 72°C.

To remove residual nucleotides, a mixture of nucleic acids was amplified and then treated with 75% alcohol. To do this, 50 µl of 75% alcohol was added to 10 µl of the mixture, and then incubated at room temperature in a dark place for 15 minutes. The mixture was then centrifuged for 15 minutes at 14,000 rpm in an Eppendorf 5427R centrifuge to precipitate the solution.

Table 1 – Primers used in work

For gene MSTN	Forward 5'-GAAACGGTCATTACCATGC-3' Reverse 5'-CATTTGGTTGCCTGAAATATG-3'.
For gene CAST	Forward 5'- TGGGGCCCAATGACGCCATCGATG-3' Reverse 5'- GGTGGAGCAGCACTTCTGATCACC-3'.

For gene FAT1 (2 pairs of primers)	1) FAT1-1 Forward 5'- GGAAAAAATACTAACGCTGAAGCACGA-3' Reverse 5'- GAGACGGTAGTTATCTGCTCCCCGA-3'. 2) FAT1-2 Forward 5'- GCGTCGGGGAGCAGATAACTAC-3' Reverse 5'- TTCAGGTTCTCTGGTTCCATAC-3'.
For gene FZD3 (2 pairs of primers)	1) FZD3-1 Forward 5'- TGGCTGTGAGTAGGATCGTC-3' Reverse 5'- ATTGCTAAAGCTGCCGTCTG-3'. 2) FZD3-2 Forward 5'- GATCCGGATTGGTGTTTTTCAGCAT-3' Reverse 5'- AGAACAAGGTTCCCTTACCTGATA-3'.

After successful precipitation, the precipitate was left at room temperature on a chemical table to dry. The precipitate was then dissolved in 10 µl of formamide, and denaturation of the deoxyribonucleic acid was carried out for 4 minutes at a temperature of 96°C. After denaturation, the tube containing the standard was left in ice for 3 minutes. The resulting DNA was then loaded onto sequencing strips and transferred to the genetic analyzer [19].

The analysis of nucleotide sequences was carried out using the Sequencing analysis 5.2 program and DNAMAN. The specificity of the nucleotide sequences was determined using the Basic Local Alignment Search Tool (BLAST) program. The obtained sequences were then analyzed by alignment using the Clustal Omega program [20].

Results and Discussion

Partial targeted sequencing of candidate genes associated with meat and wool productivity, mentioned in previously published sources [14, 21, 22], was conducted. SNP analysis was performed for genes MSTN, CAST, FAT1, and FZD3. For the MSTN gene, PCR amplification targeting the first intron was performed. The aim of the analysis was to identify two SNPs located at positions 18 (rs119102825) and 241 (rs119102826). The positions of single nucleotide polymorphisms were indicated with respect to the site and nucleotide position of the transcript of the gene (ISGC Oar_v3.1/oviAri3).

These polymorphisms are associated with such indicators as birth weight and average daily weight gain [21]. The genetic variations of the MSTN gene show differences in genotype among species and families. The GG genotype for rs119102825 was found to be common in the genome of all animals of the Edilbay breed. For the Kazak Fine-wool sheep and Akzhayik breeds, it corresponds to the TT genotype. The polymorphisms between the T and G alleles were observed in the Saryarka and Kazakh semi-coarse wool sheep, while the SNP rs119102826 showed the same genotype for all breeds. The T allele was observed for the Edilbay, Saryarka, and Kazakh semi-coarse wool sheep breeds, while the Kazak Fine-wool sheep and Akzhayik breeds corresponded to the CC genotype.

The MSTN gene functions as a growth factor in cells and also participates in the process of differentiation [23, 24, 25]. The gene is a non-coding regulator of skeletal muscle growth. The association of the gene with meat productivity is not only characteristic of sheep, but also of other animals [26].

Genetic polymorphisms in the myostatin (MSTN) gene associated with increased muscling and growth have been detected in the Madras Red, Mecheri, and Texel sheep breeds using PCR-RFLP analysis in a prior study. The MSTN gene serves as a suppressive regulator for muscle development. Consequently, the rate of muscle growth in sheep is multiplied by two when the function of MSTN is compromised [27, 28].

The obtained data for rs119102825 indicate a connection between genotype and increased meat productivity, specifically with higher birth weight. These findings are supported by associations reported by other authors [22]. Only the GG genotype in this locus is significantly associated with increased birth

weight. The SNP probability at this position for the Saryarkabreed may be explained by its greater age and higher intensity of breeding compared to other animals. The distribution of different genotypes for MSTN and CAST genes among breeds is shown in Table 2.

Table 2 – Percentage of occurrence of different genotypes for the MSTN and CAST genes among sheep breeds

Gene	Position of SNP in the gene	SNP	Percentage of occurrence of genotypes in the breed, %				
			Kazakh semi-coarse wool sheep	Akzhayik	Saryarka	Edilbay	Kazak Fine-wool sheep
MSTN	18	GG	18,9	0,8	11,9	98,2	0,0
		TG	81,1	5,5	81,2	1,0	8,8
		TT	0,0	93,7	6,9	0,8	91,2
	241	TT	95,2	4,1	89,3	92,1	6,2
		TC	0,0	0,3	0,0	0,0	0,6
		CC	4,8	95,6	10,7	7,9	93,2
CAST	383	GG	99,8	100,0	99,3	98,7	99,5
		GA	0,2	0,0	0,7	1,3	0,5
		AA	0,0	0,0	0,0	0,0	0,0

The TT genotype for the SNP rs119102826 is also associated with higher average daily weight gain for the breeds Edilbay, Saryarka, and Kazakh semi-coarse wool sheep [22]. These findings are supported by phylogenetic analysis of these breeds. The breeds most closely related to Edilbay are Saryarka and Fat-tailed semi-coarse wool sheep, which also have similar traits, such as high meat productivity and thick or coarse wool.

The CAST gene, which is associated with muscle mass growth and meat taste characteristics, represents another promising genetic mutation [29]. Mutations in the CAST gene are directly related to animal muscle mass growth and, consequently, meat productivity [30, 31]. Genetic comparisons have shown SNP monomorphism for this gene among all studied breeds. Data on the genotyping of the CAST gene is supported by data on the prevalence of GG genotypes in sheep populations around the world [21].

CAST plays an important role in muscle formation, as well as in the processes of degradation and softening of meat after slaughter. It is an inhibitor of calpain proteins, which are responsible for the degradation of myofibrillar proteins. Changes in the nucleotide sequence of the calpastatin protein

are associated with changes in its physicochemical properties, which in turn affect its structure and mechanism of action, including the control of the activity of calcium-dependent sarcoplasmic reticulum channels. This, in turn, affects the activity of calpain proteins. The process of myofibrillar breakdown in animal muscle tissue after slaughter is directly related to the characteristics of the meat [21].

Since wool is an important agricultural product and a vital source of income for sheep farming, research on genetic properties related to wool productivity is an integral part of market-oriented breeding. The value of wool is determined by properties such as the average diameter of the wool fiber and wool durability. When describing wool quality, several genes related to the qualitative characteristics of sheep wool are mentioned. Among them, the genes FAT1 and FZD3 are the most significant. Studies of published works describe a significant difference in wool quality characteristics between different sheep breeds. This difference in the expression of the FAT1 gene can be used as a marker for evaluating the wool characteristics of the breed. The product of the gene expression regulates the morphogenesis, cyclic functioning, and orientation of the hair follicles in sheep by participating in the planar cell

polarity (PCP) signaling pathway through cell adhesions. The PCP signaling pathway controls tissue polarity and cell movement by activating the RHOA signaling cascades, c-Jun N-terminal kinase (JNK), and NLK kinase. This pathway also regulates the function of other tissues, including in humans, by participating in the formation of ciliated epithelial cells [32].

Amplification with subsequent genotyping of gene FAT1 revealed the presence of four polymorphisms in exon 2. Using two pairs of primers allowed obtaining two overlapping sequences with lengths of 1692 bp and 1406 bp. Identified nucleotide polymorphisms are associated with wool productivity in sheep and hair coat type [15]. Thus, according to the results of allele analysis of the first polymorphism in the breed of Kazak Fine-wool sheep, Saryarka and Kazakh semi-fine wool sheep, the genotype GA was found. In the breed of Akzhayik, a homozygous genomic GG is possible, and in the breed of Edilbay – AA.

In addition, SNP in position 218326 is also associated with the diameter of wool fiber [15]. As a result, the genotypes were obtained: TC – for the breed of Kazak Fine-wool sheep and Akzhayik, CC – for the breeds of Edilbay and Kazakh semi-fine wool sheep, and TT – for the breed of Saryarka.

Analysis of the nucleotide of a single polar bear in position 16554 revealed the following genotypes

in populations: AA, corresponding to the Kazak Fine-wool sheep, Akzhayik as well as Saryarka, GA for the Edilbay, and GG, common in the Kazakh semi-coarse wool sheep.

For the SNP in position 16563, the genotypes are as follows: TT for Kazak Fine-wool sheep, Akzhayik and Saryarka, TG for Edilbay sheep, and GG for Kazakh semi-coarse wool sheep.

Analysis of published works on genotyped data suggests an association of SNP (c.16203) GG with higher wooliness in sheep compared to genotype GA. Genotype AA is practically not associated with wooliness. Additionally, analysis of another SNP (c.218326) shows an association not only with wooliness but also with fiber diameter. Genotype TC is characterized by finer wool compared to genotype TT, but greater wooliness compared to genotype CC [15, 33].

The prevalence of different genotypes in the FAT1 and FZD3 genes among sheep breeds is shown in Table 3, 4.

The analysis of polymorphisms at positions 16554 and 16563 demonstrates the following associations: the AA genotype for the SNP at position 16554 is associated with longer hair structure compared to the genotypes GA and GG, while the TT genotype (for the SNP at position 16563) is also associated with longer hair compared to the genotypes TG/GG [15, 33].

Table 3 – Comparison of various genotypes by the FAT1 gene among sheep breeds

Gene	In terms of SNP in the gene	SNP	The percentage of genetic similarities we encounter in the population				
			Kazakh semi-coarse wool sheep	Akzhayik	Saryarka	Edilbay	Kazak Fine-wool sheep
FAT1	16203	GG	7,7	97,3	4,1	2,8	9,7
		GA	89,3	2,5	95,9	5,2	90,0
		AA	3,0	0,2	0,0	92,0	0,3
	218326	CC	83,3	7,3	6,5	91,1	5,1
		TC	6,7	85,6	5,3	7,3	89,6
		TT	10,0	7,1	88,2	1,6	5,3
	16554	AA	5,2	82,7	87,3	2,2	93,8
		GA	3,6	12,3	9,1	97,2	6,2
		GG	91,2	5,0	3,6	0,6	0,0
	16563	TT	2,7	85,1	75,3	10,3	91,2
		TG	5,3	13,7	20,1	88,9	8,8
		GG	92,0	1,2	4,6	0,8	0,0

Table 4 – Efficiency of meetings of different genotypes regarding the FZD3 gene among sheep breeds

Gene	Position of SNP in the gene	SNP	Percentage of genotype occurrence in the breed				
			Kazakh semi-coarse wool sheep	Akzhayik	Saryarka	Edilbay	Kazak Fine-wool sheep
FZD3	101771685	TT	0,0	1,3	13,6	11,8	97,8
		TC	18,7	88,6	12,9	6,1	1,9
		CC	81,3	10,1	73,5	82,1	0,3
	101810848	AA	83,1	76,3	75,8	16,8	81,1
		AC	16,9	23,7	24,2	83,2	18,9
		CC	0,0	0,0	0,0	0,0	0,0

The FZD3 (Frizzled3) gene is expressed in many tissues and organs of mammals and is also associated with wool productivity. This is due to the involvement of the gene and its product in the Wnt signaling pathway, which regulates cell growth, differentiation, development, and homeostasis. In addition, the involvement of Frizzled family genes in the development and functioning of skin and hair cells has been demonstrated [34].

Amplification of the FZD3 gene region was carried out to analyze polymorphisms in exons 1 and 3 (Table 4). The reaction products were of lengths 184 and 170 bp, respectively. Two polymorphisms were identified. For the SNP (position 101771685) located in exon 1, the genotypes observed in the population were TT for some breeds, TC for others, and CC for all remaining breeds. For the second polymorphism (position 101810848), the AA genotype predominated in all breeds except for Edilbay, in which the AC genotype was found.

According to the research conducted by a group of Chinese scientists, it was demonstrated that there is an association of the TT genotype in position 101771685 with a smaller average diameter of the hair shaft. In addition, a polymorphism in position 101810848, represented by the AA genotype, was associated with a higher mass of straight hair fibers [17]. Therefore, the genotype of Edilbay, which does not have long and dense hairy coat, differs from all the other studied populations.

Conclusion

The conclusions of this article demonstrate the potential of sequencing candidate genes for improving agricultural production in Kazakh sheep breeding. Analysis of the MSTN, CAST, FAT1, and FZD3 genes identified variations strongly correlated with desirable traits such as meatiness, wool quality, and fat content. These results can be used for selecting animals with desired traits and further breeding, which could increase farmers' efficiency and improve product quality. This study is an important step in the development of agriculture and may be useful for practical application in the industry.

Conflict of interest

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