



Features of Holstein Cattle Bred in Kazakhstan by the Polymorphic Genes of the Somatotropin Cascade

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Abstract | The article presents the results of studying the genetic structure by *bGH*-AluI and *bIGF-1*-SnaBI polymorphisms in Holstein cows bred in Kazakhstan. It has been shown that *bGH* and *bIGF-1* genes in the Kazakh population of Holstein cows are polymorphic. By the growth hormone gene, the frequency of *bGH*-AluI^L allele is 0.82; of *bGH*-AluI^V allele – 0.18; by the gene of the insulin-like growth factor-1, the frequency of *bIGF-1*-SnaBI^A allele is 0.58; and of *bIGF-1*-SnaBI^B allele – 0.42. By all the studied polymorphic loci, the Kazakhstan population of Holstein cows is in the state of Hardy-Weinberg genetic equilibrium. The authors believe that research should be continued and the effect of genotypes of *bGH*-AluI and *bIGF-1*-SnaBI polymorphisms on milk production should be determined.

Keywords | Holstein breed, Growth hormone gene (bGH), Insulin-like growth factor gene -1 (bIGF-1), Polymorphism, Allele, Genotype

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INTRODUCTION

Currently, dairy farming is one of the most important branches of agriculture in Kazakhstan. Along with the extensive way of increasing milk production, more and more attention is attached to the intensification of the industry through improving the genetic potential of the bred animals.

Modern achievements in molecular genetics have made it possible to identify the genes associated with qualitative and quantitative traits of cattle. Identification of the preferred allelic variants of such genes will allow breeding with the use of markers at the level of DNA. One of them is the growth hormone gene, as well as the insulin-like growth factor-1.

The growth hormone is involved in regulating growth and development in animals and affects the quality parameters

of milk cows. The relationship between polymorphic variants of the growth hormone gene and milk productivity was studied by many foreign scientists. For instance, Grochowska R. and Zwierzchowski L. found a significant correlation between the presence of *bGH*-AluI^L allele and high milk yield of Holstein cows (Grochowska et al., 2001; Zwierzchowski et al., 2002; Chen et al., 2018a, 2018b).

Along with the growth hormone, the insulin-like growth factor-1 regulates growth, development, and lactation. Association of *bIGF-1* gene with traits of milk productivity is studied in various countries. For instance, Mehmannaev et al., 2010 discovered that animals of the Iranian population of Holstein cattle with *bIGF-1*-SnaBI^{AB} genotype featured high content of fat and protein in milk (Mehmannaev et al., 2010; Zhang et al., 2018; Beishova et al., 2017).

Siadkowska et al. (2006) also discovered a positive

association between *bIGF-1*-SnaBI^{AB} genotype and a high percentage of milk fat and protein in the Polish population of Holstein cows. Thus, studying the polymorphisms of growth hormone genes and insulin-like growth factor-1 is interesting from the point of determining the genetic potential of cattle by the quantitative productivity traits.

The aim of the study was analyzing the genetic structure by *bGH*-AluI and *bIGF-1*-SnaBI polymorphisms in Holstein cows bred in Kazakhstan.

MATERIALS AND METHODS

The study was performed at the Department of Molecular Genetic Studies of the Research and Innovation Center of the Kostanay State University (KSU) n.a. A. Baitursynov. The object of the study was a group of Holstein cows bred in Kazakhstan (100 animals, LLC Bek+, Kostanay region). The biological material was chosen by the personnel of the farm, and by the personnel of the Department of Molecular-Genetic Studies.

The genotypes of the animal by the polymorphic sections of *bGH* and *bIGF-1* genes were determined using the method of polymerase chain reaction, followed by an analysis of polymorphism of the restriction fragments lengths (PCR-RFLP). DNA was extracted using commercial kit PureLink Genomic DNA Mini Kit. The DNA concentration was measured on spectrophotometer Dynamica Halo DNAMaster. The polymerase chain reaction was performed on the amplifier ProFlex PCR System (Applied Biosystems). The composition of the reaction mixture was the following: water–11.8 µl, 10X buffer–2 µl, dNTP (2.5 mM)–0.4 µl, MgCl₂ (25 mM)– 2 µl, primer F (10 pM)–1 µl, primer R (10 pM)–1 µl, and Taq DNA Polymerase (5U/µl)–0.3 µl. The primers sequences and their annealing temperature are shown in Table 1.

The obtained amplicates of *bGH* and *bIGF-1* genes were restricted using the SnaBI and AluI (Thermo Scientific) restriction endonucleases. After incubation, the obtained fragments were separated in 3% agarose gel (Invitrogen). To visualize the results of electrophoresis, the Quantum 1100 gel documentation system (Vilber Lourmat) was used.

bGH-AluI polymorphism is contingent on transition C→G, resulting in amino acid leucine replacement with valine in the protein sequence. The allele recognized by the enzyme contains nucleotide C and is labeled as *bGH*-AluI^L. If the G nucleotide is present, the restriction segment disappears; such an allele is denoted as *bGH*-AluI^V (Figure 1).

Polymorphism of the nucleotide sequence of *bIGF-1* gene in area P1 of the promotor region is identified as T→C

transversion. Fragment of *bIGF-1* gene with the length of 249 b.p. is amplified. The length of fragments after the restriction is 223 and 26 b.p. (Figure 2).

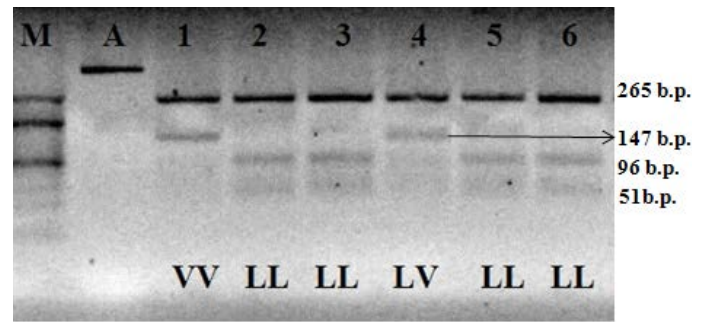


Figure 1: Electrophoretogram of the amplification products and restriction of the fragment of *bGH* gene; 1–6 hole numbers; A). Amplificate of the polymorphic section of gene *bGH*; M). The marker of molecular masses O' RangeRuler 20 bp DNA Ladder (Thermo Scientific); LL, LV and VV–relevant genotypes.

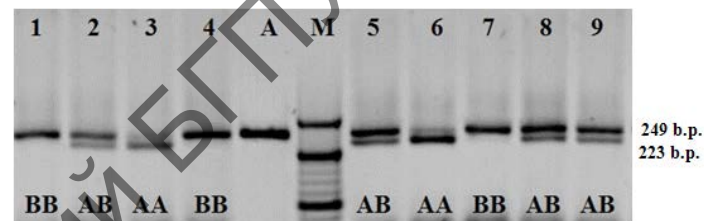


Figure 2: Electrophoretogram of the amplification products and restriction of the fragment of *bIGF-1*-SnaBI gene; 1–9 hole numbers; A). Amplificate of the polymorphic section of gene *bIGF*; M) The marker of molecular masses O' RangeRuler 20 bp DNA Ladder (Thermo Scientific); AA, AB and BB–relevant genotypes.

Based on the obtained data, the expected frequency of genotypes and alleles was calculated according to the genetic equilibrium of the population by Hardy-Weinberg using the χ^2 method.

RESULTS AND DISCUSSION

After the PCR-RFLP analysis, distribution of animals by genotypes of the locus of the growth hormone gene (*bGH*) was the following: 67 cows had the homozygous *bGH*-AluI^{LL} genotype; 29 cows had the heterozygous *bGH*-AluI^{LV} genotype, and four cows had the homozygous *bGH*-AluI^{VV} genotype. With that, the frequency of *bGH*-AluI^L allele was 0.82, of *bGH*-AluI^V allele–0.18 (Table 2).

Many scientists in the near and far abroad countries have analyzed the genetic structure of *bGH*-AluI polymorphism in various populations of cattle (Table 3). The frequency of occurrence of *bGH*-AluI^V allele in various cattle breeds varies between 0.064 and 0.280 for dairy cattle, and between 0.174 and 0.358 for beef breeds

Table 1: The primers sequence for amplification of polymorphic sections of the growth hormone gene and insulin-like growth factor-1.

Gene	Primers sequence	Primers annealing temperature	Amplificate size, pairs of nucleotides (b.p.)	Reference
bGH	F: 5'-ccgtgtctatgagaagc-3'; R: 5'-gttcttgagcagcgct-3'	60° C	451 b.p.	(Lucy et al., 1993)
bIGF-1	F:5'-attacaaagctgctgcccc-3'; R:5'-accttaccgtatgaaaggaatatacgt-3'	62° C	249 b.p.	(Skinkyte et al., 2005).

Table 2: Frequencies of alleles and genotypes of *bGH*-AluI polymorphism in the Holstein cattle bred in Kazakhstan.

Allele	Observed allele frequencies	Relative allele frequencies	Genotype	Number of genotypes	Frequencies of genotypes
<i>bGH</i> -AluI ^L	163	0.82 ± 0.004	<i>bGH</i> -AluI ^{LL}	67	0.67
<i>bGH</i> -AluI ^V	37	0.18 ± 0.004	<i>bGH</i> -AluI ^{LV}	29	0.29
			<i>bGH</i> -AluI ^{VV}	4	0.04
Total				100	1

Table 3: Distribution of the relative frequencies of the alleles of *bGH*-AluI polymorphism in various populations of Holstein cattle.

Breed	Relative frequencies of alleles		Frequencies of genotypes			Country	Author, reference
	<i>bGH</i> -AluI ^L	<i>bGH</i> -AluI ^V	<i>bGH</i> -AluI ^{LL}	<i>bGH</i> -AluI ^{LV}	<i>bGH</i> -AluI ^{VV}		
Dairy cattle breeds							
Holstein (n = 134)	0.936	0.064	0.87	0.13	0	Iran	(Lucy et al., 1993)
Holstein	0.93	0.07	0.85	0.15	0	USA	(Sadeghi, et al., 2008)
Holstein-Friesian (n = 19)	0.92	0.08	0.84	0.16	0	New Zealand	(Hartatik et al., 2016)
Holstein-Friesian (n = 43)	0.90	0.10	0.79	0.21	0	Australia	(Hartatik et al., 2016)
Black and White (n = 250)	0.72	0.28	0.495	0.625	0.180	Russia	(Dolmatova and Ilyasov, 2011)
Beef cattle breeds							
Kazakh white-headed (n = 296)	0.826	0.174	0.67	0.31	0.02	Kazakhstan	(Beishova, et al., 2018)
Angus (n = 116)	0.681	0.319	0.102	0.434	0.464	Ukraine	(Fedota, et al., 2016)
Auliekol (n = 284)	0.667	0.333	0.45	0.44	0.11	Kazakhstan	(Beishova, et al., 2018)
Limousine (n = 100)	0.642	0.358	0.461	0.363	0.176	Poland	(Dybus, et al., 2002)

Table 4: Frequencies of alleles and genotypes of *bIGF-1*-SnaBI polymorphism in the Holstein cattle bred in Kazakhstan.

Allele	Observed allele frequencies	Relative allele frequencies	Genotype	Number of genotypes	Frequencies of genotypes
<i>bIGF-1</i> -SnaBI ^A	116	0.58 ± 0.005	<i>bIGF-1</i> -SnaBI ^{AA}	18	0.18
<i>bIGF-1</i> -SnaBI ^B	84	0.42 ± 0.005	<i>bIGF-1</i> -SnaBI ^{AB}	48	0.48
			<i>bIGF-1</i> -SnaBI ^{BB}	34	0.34
Total				100	1

(Lucy et al., 1993; Sadeghi et al., 2008; Hartatik et al., 2015; Dolmatova and Ilyasov, 2011; Beishova et al., 2018; Fedota et al., 2016; Dybus et al., 2002) The obtained results correspond to the published data, and are within the frequency limits for the alleles obtained by various authors for both milk and beef breeds.

Table 5: Distribution of the relative frequencies of the alleles of *bIGF-1*-SnaBI polymorphism in various populations of Holstein cattle.

Breed	Relative allele frequencies		Frequencies of genotypes			Country	Author, reference
	<i>bIGF-1</i> -SnaBI ^A	<i>bIGF-1</i> -SnaBI ^B	<i>bIGF-1</i> -SnaBI ^{AA}	<i>bIGF-1</i> -SnaBI ^{AB}	<i>bIGF-1</i> -SnaBI ^{BB}		
Dairy cattle breeds							
Holstein-Friesian (n = 42)	0.63	0.37	0.33	0.59	0.07	Uruguay	(Nicolini et al., 2013)
Holstein-Friesian (n = 28)	0.52	0.48	0.28	0.46	0.25	New Zealand	(Nicolini et al., 2013)
Holstein-Friesian (n = 662)	0.52	0.48	0.29	0.47	0.24	Poland	(Siadkowska et al., 2006)
Holstein (n = 282)	0.438	0.562	0.159	0.557	0.284	Iran	(Mehmanna et al., 2010)
Beef cattle breeds							
Korean	0.72	0.28	0.586	0.264	0.150	Korea	(Chung and Kim, 2005)
Charolais (n = 68)	0.46	0.54	0.208	0.500	0.292	Mexico	(Reyna, et al., 2010)
Canchim (n = 30)	0.35	0.65	0.133	0.433	0.434	Brazil	(Curi et al., 2005)
Charolais (n = 43)	0.26	0.74	0.070	0.372	0.558	Mexico	(Reyna, et al., 2010)
Beefmaster (n = 25)	0.03	0.97	0	0.067	0.933	Mexico	(Reyna, et al., 2010)
Nelore (n = 79)	0	1	0	0	1	Brazil	(Curi et al., 2005)

Table 6: Distribution of the frequencies of the genotypes of the somatotropin cascade polymorphic genes in the populations of Holstein cattle bred in Kazakhstan.

Polymorphism	Genotype	n observed	n expected	χ^2
<i>bGH</i> -AluI n = 100	<i>bGH</i> -AluI ^{VV}	4	3	0.15
	<i>bGH</i> -AluI ^{LV}	29	30	
	<i>bGH</i> -AluI ^{LL}	67	66	
<i>bIGF-1</i> -SnaBI n = 100	<i>bIGF-1</i> -SnaBI ^{BB}	34	34	0.02
	<i>bIGF-1</i> -SnaBI ^{AB}	48	49	
	<i>bIGF-1</i> -SnaBI ^{AA}	18	18	

As a result of the DNA diagnostics of the Kazakhstan population of Holstein cows by the polymorphic locus of *bIGF-1* gene, it has been found that out of 100 animals, 18 had *bIGF-1*-SnaBI^{AA} genotype, 48 cows had *bIGF-1*-SnaBI^{AB} genotype, and 34 cows had *bIGF-1*-SnaBI^{BB} genotype. With that, the frequency of *bIGF-1*-SnaBI^A allele was 0.58, and that of *bIGF-1*-SnaBI^B allele—0.42 (Table 4).

A number of studies have been devoted to defining the polymorphism of the insulin-like growth factor-1 gene in cattle (Table 5) (Mehmannavaz et al., 2010; Siadkowska et al., 2006; Nicolini et al., 2013; Chung and Kim, 2005; Reyna, et al., 2010; Curi et al., 2005).

Studying the SnaBI- polymorphism of *bIGF-1* gene showed that in Holstein and Holstein-Friesian cattle breeds, the frequency of *bIGF-1*-SnaBI^A allele was 0.438 – 0.630, and the frequency of *bIGF-1*-SnaBI^B allele— 0.370 – 0.562, respectively. In the beef breeds (Korean, Charolais, Canchim, Beefmaster, Nelore), the frequency of *bIGF-1*-SnaBI^A allele was in the range between 0 and 0.72, while the frequency of *bIGF-1*-SnaBI^B allele—between 0.28 and 1. In the study of the authors, the frequency of *bIGF-1*-SnaBI^A and *bIGF-1*-SnaBI^B alleles was medium, and amounted to 0.58 and 0.42, respectively.

The authors have also analyzed the conformity of the observed frequencies of the genotype to the theoretically

expected equilibrium distribution in accordance with the law of Hardy-Weinberg (Table 6). The significance of the observed deviations was assessed using criterion χ^2 .

Table 6 shows that for Holstein cattle, for *bGH*-AluI and *bIGF-1*-SnaBI polymorphisms, the correspondence of the observed frequencies of the genotype to the ones theoretically expected is observed. This is evidence of the fact that the population of Holstein cows bred in Kazakhstan is genetically stable, and no natural and artificial selection is observed in it. Next, the authors plan to study the polymorphism of cattle *LTF* and *MBL1* genes, and their association with economically useful traits.

CONCLUSION

Thus, the authors have found the following. The rare to common alleles ratio for *bGH*-AluI polymorphism coincides with the populations of Holstein cattle bred in various countries. The work has established that, like in other works, the rare one is *bGH*-AluI^V allele. This observation suggests that allele of *bGH*-AluI^L may provide selective advantages on the background of feeding peculiarities or climatic conditions. Allele frequencies of *bIGF-1*-SnaBI polymorphism, which were found in the population of Holstein cows bred in Kazakhstan, correlate with those established by other researchers. Unlike in the dairy ones, in the meat breeds, the frequency of *bIGF-1*-SnaBI^B allele is higher; it is possible since along with the development of meat qualities, animals of this breed with excellent genotype were selected.

The results of analyzing the distribution of genotypes show that the observed genotype frequencies in the local Kazakhstan population of Holstein cows by *bGH*-AluI and *bIGF-1*-SnaBI polymorphisms coincide with those expected according to the law of Hardy-Weinberg, which indicates the absence of artificial selection in the population.

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AUTHORS CONTRIBUTION

All authors contributed equally.

The authors declare no conflicts of interest.

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