

Part 1. Biotechnology

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SNP L127V OF GROWTH HORMONE GENE IN BREEDING HERD OF ABERDEEN ANGUS IN KHARKIV REGION, EASTERN UKRAINE

¹ Fedota O. M., ² Ruban S. Yu., ³ Lysenko N. G., ⁴ Kolisnyk A. I., ⁵ Goraichuk I. V., ⁶ Tyzhnenko T. V.

¹ V. N. Karazin Kharkiv National University, Kharkiv, Ukraine, e-mail: afedota@mail.ru

² Institute of Animal Breeding and Genetics nd. a. M. V. Zubets of NAAS, Chubynske, Ukraine

³ V. N. Karazin Kharkiv National University, Kharkiv, Ukraine

⁴ PE 'Agrofirma Svitanok', Novoselivka, Ukraine

⁵ National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine

⁶ V. N. Karazin Kharkiv National University, Kharkiv, Ukraine

Summary. Bovine growth hormone (*bGH*) effects on animal growth and metabolism. Therefore, it plays a vital role in regulation of body weight, fertility and lactation performance of cattle. This enables *bGH* gene to be used as a candidate marker for improving growth, meat or milk production and for marked-assisted selection programs of cattle too. The aim of this study is analyzing the polymorphic variant L127V of *bGH* gene in Aberdeen-Angus bred in Kharkiv region and comparing of obtained result with commercial herds from other countries.

PCR-RFLP methods were set up for the SNP genotyping. Testing deviation from the Hardy-Weinberg equilibrium was performed using Pearson's chi-squared test. Pearson's correlation coefficient *r* was used to measure the strength of association between two characteristics.

The allele and genotype frequencies of SNP L127V (rs41923484; g.2141C>G) are: L — 0.319 and V — 0.681; LL — 8.6%, LV — 46.6% and VV — 44.8% (n=58), population is in Hardy-Weinberg equilibrium. The studied population is close to beef herds of Russian selection. It was found a positive correlation of L-allele with birth weight within the moderate climatic zone (*r*=0.93).

Keywords: Aberdeen-Angus breed, growth hormone gene, SNP L127V

Introduction. Bovine growth hormone (*bGH*) belongs to a family of somatotrophic hormones. It is a single polypeptide chain with 190 or 191 amino acids and molecular weight 22 kDa (Salces et al., 2011). Growth hormone has an effects on growth and metabolism by interacting with specific receptor on the surface of target cells. *bGH* gene is located in 19 BTA and consists of five exons separated by four introns (Hadi et al., 2015). *bGH* plays a vital role in regulation of body weight, fertility and lactation performance of cattle. Therefore, SNPs in *bGH* gene can be used as predictors of growth, meat or milk production traits for supporting marked-assisted selection programs in cattle.

To date there are following SNPs of *bGH* gene investigated in cattle: four (253 C>T, 303 C>T, 502 C>T, and 559 G>A) in the promoter, one (679 C>T) in exon 1, one (1,692 T>C) in intron 3 four (2141 C>G, 2258 C>T, 2277 C>T, and 2291 A>C) in exon 5 (Lee et al., 2013) and one (C/T in 40.5 Mb locus) in intron 4, which could be used to differentiate humped (*B. indicus*) from humpless (*B. taurus*) cattle (Thomas et al., 2007).

Considering a multiple effect of *bGH* on growth and lactation processes SNPs of *bGH* gene was studied

both in beef and dairy breeds: Aberdeen Angus — in Japan, USA, Brazil (Chikuni et al., 1991; Ge et al., 2003; Vasconcellos et al., 2003), Simmental and Hereford — in Lithuania (Krasnopiorova et al., 2012), Charolais — in Lithuania, Brazil (Krasnopiorova et al., 2012; Kemenes et al., 1999; Regitano et al., 1999), Limousin — in Lithuania, Indonesia (Krasnopiorova et al., Hartatik, 2013), Holstein — in Japan, Germany, Hungary, Poland, Lithuania, Iran (Chikuni et al., 1991; Hradecka et al., 2008; Balogh et al., 2009; Krasnopiorova et al., 2012; Hadi et al., 2015), Jersey — in Italy, Poland (Dario et al., 2008; Komisarek et al., 2011), Brangus — in Mexico (Thomas et al., 2007), Nellore — in Brazil (Kemenes et al., 1999), as well as endemic cattle breeds — in Philippines (Salces et al., 2011) and Korea (Lee et al., 2013). These *bGH* SNPs are associated with birth weight, weight gain, constitution, milk yield, fat, protein, fertility.

Studies of *bGH* SNP L127V in Ukrainian breeds were carried out in small populations of Aberdeen-Angus, Southern Beef, Polissian Beef (Kopylova et al., 2009; Kostenko, Starodub, 2011), Ukrainian Grey (Podoba et al., 2009; Mokhnachova et al., 2016), Volinian Beef (Bochkov et al., 2009), Holstein (Kopylova et al., 2009;

Hyl et al., 2011; Nekrasov et al., 2016), Ukrainian Black-and-White Dairy (Nekrasov et al., 2016), Ukrainian Red-and-White Dairy and Ukrainian Red Dairy (Kopylova et al., 2009).

The purpose of the study was to conduct a comparative analysis of the alleles and genotypes frequencies distribution for SNP *L127V* in the Aberdeen-Angus breeding herd in Kharkiv region and to compare obtained result with similar parameters of commercial herds from other countries.

Material and methods. The study object was Aberdeen-Angus breeding herd (n=58; cows: n=52, bulls: n=6) bred at PE 'Agrofirma Svitanok' (Kharkiv region, Ukraine). Evaluation of growth dynamics was conducted via the control weighing at birth and at the age of 8, 12, 15, 18 months, and 2, 3, 4, 5 years.

DNA was extracted from blood samples using DNA extraction kits 'Diatom DNA Prep 100' (Isogene, Russian Federation). PCR-RFLP methods were set up for the SNP genotyping using primer pairs (Lee et al., 2013) and restriction endonuclease *AluI* ('Fermentas', Lithuania). The digested fragments were electrophoresed on 2.0% agarose gel.

The genetic distance between the studied population and data known from the literature was determined according to Nei (1972). The deviation of allele frequencies from Hardy-Weinberg equilibrium was tested using Pearson's chi-squared test. Normality test assessment was used for data distribution. Pearson's correlation coefficient *r* was used to measure the strength of association between two characteristics. Means for more than two groups were compared by ANOVA. The statistical hypotheses were tested on the significance level of 0.05 (Atramentova, Utevskaia, 2008).

Results. According to the literature the preferred allele is *L-allele*, as it is associated with higher birth weight (Lee et al., 2013; Thomas et al., 2007) and mature weight, marbling (Gill et al., 2009).

Allele and genotype frequencies of SNP *L127V* and its deviation from the Hardy-Weinberg equilibrium for studied Aberdeen-Angus herd are given in Table 1.

Table 1 – Allele and genotype frequencies of SNP *L127V* in the Aberdeen-Angus herd in Kharkiv region

Parameter	<i>L127V</i>		
	<i>L</i>	<i>V</i>	
Allele frequencies			
Cows (n=52)	0.333	0.667	
Bulls (n=6)	0.167	0.833	
Total (n=58)	0.319	0.681	
Genotype frequencies	<i>LL</i>	<i>LV</i>	<i>VV</i>
Cows (n=52)			

<i>n_{act.}</i>	5	25	22
%	9.6	48.1	42.3
<i>n_{exp.}</i>	5.9	23.2	22.9
%	11.3	44.6	44.1
Statistics	$\chi^2_{act.} = 0.16; p > 0.05$		
Bulls (n=6)			
<i>n_{act.}</i>	0	2	4
%	0.0	33.3	66.7
<i>n_{exp.}</i>	0.2	1.7	4.1
%	2.8	27.8	69.4
Statistics	$\chi^2_{act.} = 0.20; p > 0.05$		
Total (n=58)			
<i>n_{act.}</i>	5	27	26
%	8.6	46.6	44.8
<i>n_{exp.}</i>	5.9	25.2	26.9
%	10.2	43.4	46.4
Statistics	$\chi^2_{act.} = 0.15; p > 0.05$		

Note: $df=2, \Sigma\chi^2_{st.0.05}=5.99$.

Data given in Table 1 suggest that studied population was found to be in equilibrium state. Consequently, there are no evidence of increasing preferred allele frequency without targeted selection.

Three genotype groups separated by SNP *L127V* were compared via body weight dynamics from birth to the age of five years (Table 2).

Table 2 – Body weight dynamics in Aberdeen-Angus by SNP *L127V*, $x \pm s_x$

Age	Weight, kg		
	<i>L127V</i>		
	<i>LL</i>	<i>LV</i>	<i>VV</i>
Birth	35.2±1.3*	30.4±1.0*	29.9±0.9*
Average daily gain, kg/day	0.763±0.54	0.771±0.15	774±19
8 months	220.8±15.3	213.3±4.7	208.7±4.1
12 months	288.8±18.6	280.4±4.4	272.8±6.0
15 months	342.0±19.8	324.0±4.9	318.9±4.4
18 months	380.8±24.9	369.1±6.2	362.6±4.1
2 years	421.4±19.0	416.2±6.6	413.1±7.1
3 years	460.3±22.8	446.9±8.5	446.8±10.3
4 years	505.0±18.1	488.7±9.8	479.9±10.9
5 years	588.0±40.0	557.0±19.8	579.8±21.8

Note: $x \pm s_x$ — mean ± standard error; * — differences are significant at $p < 0.05$

Table 3 – Genotype and allele frequencies at SNP *L127V* in the beef cattle populations by climate zones (longitudinal length)

Breed	Breed characteristics	Country	Longitudinal length	n	Genotypes, %				Alleles			HWE	Reference	D
					LL	LV	VV	L	L	V				
Beef or beef-dairy breeds														
Aberdeen-Angus	CLM=500–700 kg BLM=750–1000 kg BW=16–25 kg	Ukraine	49° N	58	8.6	46.6	44.8	0.319	0.681	0.681	E	Present study	—	
		Ukraine	49° N	9	11.1	55.6	33.3	0.389	0.611	0.611	E	Kostenko et al., 2011	0.005	
		Ukraine	49° N	10	60.0	40.0	0.0	0.800	0.200	0.200	E	Kopylova et al., 2009	0.428	
		USA	40° N	468	34.4	55.1	10.5	0.620	0.380	0.380	DE	Geet et al., 2003	0.163	
		Japan	35° N	6	33.3	50.0	16.7	0.590	0.410	0.410	E	Chikuniet al., 1991	0.130	
		Brazil	11° S	52	—	—	—	0.770	0.230	0.230	—	Vasconcelos, 2003	0.378	
Auliekol	CLM=540–560 kg BLM=950–1050 kg	Kazakhstan	48° N	50	—	—	—	0.640	0.360	0.360	—	Beyshova et al., 2016	0.188	
Charolais	CLM=600–700 kg BLM=1000–1200 kg BW=40–45 kg	Lithuania	55° N	—	75.0	20.0	5.0	0.850	0.150	0.150	E	Krasnopiorova et al., 2012	0.516	
		Brazil	11° S	32	—	—	—	0.720	0.280	0.280	—	Kemenes et al., 1999	0.299	
		Brazil	11° S	36	—	—	—	0.736	0.264	0.264	—	Regitanoet al., 1999	0.323	
Hanwoo	CLM=550–600 kg	South Korea	36° N	231	84.8	11.7	3.5	0.907	0.093	0.093	E	Lee et al., 2013	0.622	
Hereford	CLM=650–850 kg BLM=900–1350 kg BW=28–35 kg	Lithuania	55° N	—	90.0	0.0	10.0	0.900	0.100	0.100	DE	Krasnopiorova et al., 2012	0.608	
		Japan	35° N	10	70.0	20.0	10.0	0.800	0.200	0.200	E	Chikuni et al., 1991	0.428	
Indigenous Philippine Cattle	CLM=300–400 kg	Philippines	13° N	55	20.0	45.0	35.0	0.430	0.570	0.570	E	Salces et al., 2011	0.017	
Kalmyk	CLM=420–480 kg BLM=750–950 kg BW=20–25 kg	Russia	60° N	60	1.7	20.0	78.3	0.117	0.883	0.883	E	Sulimova et al., 2011	0.057	
		Russia	60° N	79	5.1	31.6	63.3	0.209	0.791	0.791	E	Sulimova et al., 2011	0.021	
Kazakh White-headed	CLM=540–580 kg BLM=850–950 kg BW=27–30 kg	Lithuania	55° N	—	58.3	11.1	30.6	0.639	0.361	0.361	DE	Krasnopiorova et al., 2012	0.186	
		Indonesia	5° S	6	66.67	33.33	0.0	0.830	0.170	0.170	E	Hartatik et al., 2013	0.480	
Limousin	CLM=550–600 kg BLM=1000–1100 kg BW=34–42 kg	Indonesia	5° S	81	81.5	18.5	0.0	0.910	0.090	0.090	E	Hartatik et al., 2013	0.627	

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Limousin-Ongole crossbred	—	Indonesia	5° S	56	78.6	21.4	0.0	0.890	0.110	E	Hartatik et al., 2013	0.590
Madura	BLM=250–300 kg	Indonesia	5° S	65	100.0	0.0	0.0	1.000	0.000	—	Hartatik et al., 2013	0.802
Mongolian cattle (beef-dairy)	—	Russia	60° N	47	0.0	25.0	75.0	0.125	0.875	E	Sulimova et al., 2011	0.053
Polissian Beef	CLM=550–600 kg BLM=900–1000 kg BW=28–32 kg	Ukraine	49° N	13	69.2	23.1	7.7	0.807	0.193	E	Kostenko et al., 2011	0.440
Simmental	CLM=550–900 kg BLM=850–1300 kg BW=34–36 kg	Lithuania	55° N	—	50.0	12.5	37.5	0.563	0.438	DE	Krasnopiorova et al., 2012	0.102
Southern Beef	CLM=550–650 kg BLM=950–1100 kg BW=25–30 kg	Ukraine	49° N	8	87.5	12.5	0.0	0.937	0.063	E	Kostenko et al., 2011	0.679
		Ukraine	49° N	21	61.9	38.1	0.0	0.810	0.190	E	Kopylova et al., 2009	0.445
Ukrainian Grey	CLM=450–500 kg BLM=800–850 kg BW=27–30 kg	Ukraine	49° N	84	98.0	2.0	0	0.980	0.020	E	Mokhnachova et al., 2016	0.763
		Ukraine	49° N	51	—	—	—	0.706	0.294	—	Podoba et al., 2009	0.278
Volinian Beef (breeding herd)	CLM=500–550 kg	Ukraine	49° N	27	56.0	44.0	0.0	0.778	0.222	E	Bochkov et al., 2009	0.391
Volinian Beef (production herd)	BLM=950–1050 kg BW=28–32 kg	Ukraine	49° N	41	34.0	44.0	22.0	0.561	0.439	E	Bochkov et al., 2009	0.101
Wagyu	CLM=450–500 kg	Japan	35° N	25	24.0	40.0	36	0.440	0.560	E	Chikuni et al., 1991	0.020
Zeboid hybrid	—	Russia	60° N	95	3.2	37.9	58.9	0.222	0.778	E	Sulimova et al., 2011	0.018

Note: CLW — cow live weight, BLW — bull live weight, BW — birth weight; HWB — Hardy-Weinberg equilibrium; E — equilibrium, DE — disequilibrium; D — Nei's genetic distance.

The differences between the groups in birth weight were 4.8–5.2 kg or 14.6–16.1% ($p < 0.05$). Group *LL* was superior to *LV* or *VV* growth at each time point, although the growth dynamics between groups was comparable. The differences in the live weight decreased over time and were 3–25 kg or 1–5% between *LL* and *LV* groups, and 10–25 kg, or 2–7% between *LL* and *VV* groups.

Our findings are consistent with the results obtained by Lee et al. (2013) and Thomas et al. (2007): birth weight of *LL*-animals was eventually greater. In beef cattle *L-allele* was associated with a higher body weight and marbling score (Gill et al., 2009).

Literature data on beef cattle populations in the world are summarized in Table 3.

Mostly, literature data suggest that analyzed populations were in a state of equilibrium, except Simmental, Limousine, Hereford in Lithuania and Aberdeen-Angus in USA. Observed disequilibrium may be a consequence of an unbalanced number of heterozygous animals.

The different breeds in Russia show a low frequency of allele *L* (Sulimova et al., 2011), presumably, to be determined by long-term selection in indigenous isolated populations. Alternatively, the *L-allele* frequency in different breeds within equatorial zone is high (Hartatik et al., 2013, Lee et al., 2013). Smaller animal size, being characteristic for this climate zone according to the Bergman's rule, can therefore support a size increasing due to long-term selection, resulting to *L-allele* frequency increase.

Since the *L-allele* is associated with a higher body weight, correlation analysis for this allele frequency vs. different species birth and mature weight within a single

zone climate was carried out. A positive correlation of *L-allele* with birth weight ($r = 0.93, p < 0.05$) was established for different breeds within the moderate climatic zone. In general, Ukrainian breeds are similar for mature weight (the differences do not exceed 50–100 kg) and the *L-allele* frequency. The last observation is supported by our data on growth dynamics, where effect of *bGH* is mostly pronounced before till two-year age.

There was a trend to increasing *L-allele* frequency with decreasing latitude for Limousine and Aberdeen-Angus breeds, while the reverse trend was observed for Charolais and Hereford breeds. That is the last larger breeds (average CLW=650 kg, BLW=1200 kg) in the warmer climate show decrease in *L-allele* frequency being associated with decrease of animal body size in warm climate. Smaller breeds, Limousine and Aberdeen-Angus, (up to CLW=600 kg, BLW=800–1000 kg) demonstrate more stable allele frequencies with a tendency to a slight increase. Regardless of the region, the breed (being introduced worldwide) demonstrates stable *L-allele* frequency to be typical for the breed.

Nei's genetic distances showed that the closest to the studied population was the Ukrainian Aberdeen-Angus population (Kostenko et al., 2011) and some breeds of Russian selection (Sulimova et al. 2011). The highest differences, over 0.65, are observed for breeds of Ukrainian selection — Southern Beef and Ukrainian Grey, as well as for Madura (Hartatik et al., 2013).

Conclusion. The preferred allele frequency of SNP *L127V* was 0.319. The studied population is close to beef herds of Russian selection. It was found a positive correlation of *L-allele* with birth weight within the moderate climatic zone ($r = 0.93$).

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