

GENETIC CHARACTERIZATION OF THE MIRGOROD PIG BREED, OBTAINED BY ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISMS OF GENES

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Received May 16, 2019 / Received June 21, 2019 / Accepted July 19, 2019

Aim. To determine genetic characteristics of the Mirgorod pig breed by analysis of 25 SNPs of 22 genes and to conduct the associative analysis of genes *MC4R* (SNP c.1426 G > A), *LEP* (SNP g.2845 A > T), *GH* (*BsuRI*-polymorphism), *CTSF* (SNP g. 22 G > C) with productive traits of animals. **Methods.** Blood samples of pedigree Mirgorod pigs, bred at SI «Experimental farm named after Decemberists», Poltava region, were used for the studies. DNA genotyping was performed by PCR-RFLP and TaqMan. **Results.** Specific features of the breed were determined in terms of gene allele frequencies, high level of genetic variability ($H_e = 0.326$) and allelic diversity (mean number of alleles per locus – 1.96). The *KPL2/m* allele that causes genetic anomaly of ISTS is absent among investigated Mirgorod pigs, and the recessive *RYR1* g.1843T allele, responsible for stress sensitivity of pigs, occurs at a low frequency (0.04). Unlike other breeds, a relatively high frequency of the minor allele g.15A (0.16) of *CTSK* and polymorphism of the *LEP* gene (SNP g.3996 T > C) ($H_e = 0.455$) was observed. Statistically significant associations of polymorphisms have been established: *MC4R* (SNP c.1426 G > A) with age of gaining 100 kg, the thickness of backfat and the Eye Muscle Area, *GH/BsuRI* with the age of gaining 100 kg, and *CTSF* (SNP g. 22G > C) with Eye Muscle Area. There was a trend of statistically significant differences between groups of pigs with different genotypes of *LEP* (SNP g.2845 A > T) and the thickness of the backfat ($p = 0.09$). **Conclusions.** It is reasonable to carry out the restoration of the gene pool of the Mirgorod pig breed, taking into account the SNPs of the studied genes and their associations with the productive traits. It is expedient to give preference to pigs with SNP genotypes c.1426 *MC4R GA*, *MC4R AA*, g. 22 *CTSF CC*, g.2845 *LEP TT* for breed reproduction.

Keywords: Mirgorod pig breed, genetic characteristics, QTL, SNP.

DOI: <https://doi.org/10.15407/agrisp6.02.047>

Introduction. One of negative consequences, resulting from the distribution of industrial technology of animal production, has become the decrease in genetic diversity of agricultural breeds. In pig breeding, the main bulk of the global livestock is presented with three breeds – Large White (Yorkshire), Landrace and Duroc. In general, these breeds are more productive and better adapted to the conditions of industrial technology compared to local ones. However, local breeds, most of which are at the verge of extinction, have some unique traits, which could be useful in

case of changes in economic conditions and the loss of which could result in depauperation of the global gene fund of animals. Among these traits of local breed pigs, one can distinguish high adaptation and resistance abilities, conformational and performance stability, high viability, plasticity, plain feeding, excellent reproduction and maternal characteristics, high product quality [1–3].

Mirgorod pig breed is one of such local breeds, which has come to the verge of extinction, regardless of unique useful characteristics. This breed was approved in 1940. It was obtained via complicated reproductive crossing of local short-eared pigs with English breeds – Berkshire, Tamworth, Middle Large, and Large White. Long-term mass selection while breeding local pigs of

Poltava region «within themselves» played a great role in the process of establishment [4, 5].

It is well known that the content of intramuscular fat is one of the main factors, impacting the tenderness and energy value of meat. According to the data, presented in the articles [6–8], muscular tissue of purebred Mirgorod pigs contains extra-high level of intramuscular fat (6.56 %) and is remarkable for a low content of proteins (20.33 %). Along with low content of moisture in the meat of this breed, this trait conditions its excellent taste qualities. In addition to the abovementioned, another specific feature of this breed is its ability to use pastures and a considerable percentage of non-concentrated fodder in the ratio [9]. This is conditioned by the fact that Mirgorod breed was established on the basis of local pigs, which had been better adapted to digesting fibers.

Until recently, the main problem in preserving the gene fund of Mirgorod breed was its low number of livestock and, as a result, forced use of in-breeding in its selection.

However, the situation was not critical. The studies, conducted in 2010–2014, noted a low degree of in-breeding, and thus, a possibility of purebred selection without the risk of inbred depression [9]. This conclusion is also confirmed by the analysis of microsatellite loci of Mirgorod breed, which determined that its genetic variability was high, but lower ($N_a = 2.92$; $H_o = 0.382$; $FIS = 0.178$), compared to three other Ukrainian local pig breeds – Ukrainian Meat, Ukrainian White Steppe and Ukrainian Spotted Steppe (mean $N_a = 5.00–8.42$; $H_o = 0.549–0.668$; $FIS = 0.027–0.066$) [10].

However, the spreading of African swine fever (ASF) in Ukraine has become the highest threat for breed preservation. According to the data of the World Organization for Animal Health [11], since the beginning of 2016, 123 thousand pigs were eliminated in Ukraine due to the outbreak of ASF, not counting the ones, eliminated in private households.

In August 2018, the only purebred herd of Mirgorod pig breed in the world, which belonged to SI “EF named after Decemberists” was eliminated too, due to the outbreak of ASF. As of December 2018, only 9 animals of Mirgorod breed remained alive in the experimental station of the Institute of Swine Production and Agroindustrial Production, NAAS, and 14 pigs – in private households. In addition, the genetic material bank of the Institute of Animal Breeding and Genetics, NAAS, keeps the sperm of 5 Mirgorod breed boars,

obtained in 2016. Therefore, there are some prerequisites for restoration of Mirgorod breed. Here, one of possible constituents of solving this task is using the genetic material of Belorussian Black-and-Spotted pig breed, the selection scheme of establishing of which was similar to the scheme of establishing the Mirgorod breed, and which is close to the latter by the phenotype features. Another possibility is the involvement of Poltava Meat breed, which was created using Mirgorod breed sows and inherited specific mitochondrial haplotype from the latter. It is important for the population of the restored breed to be maximally genetically similar to the initial Mirgorod breed, which is practically non-existent at present.

Taking this fact into consideration, it is relevant to obtain genetic characteristics of the Mirgorod breed using the information about single nucleotide polymorphisms. Genetic characterization is supplemented with the associative analysis of the relationship between some SNPs and the manifestation of productive features of purebred pigs.

MATERIALS AND METHODS

The population studies involved the use of blood samples of pedigree Mirgorod pigs, bred at SI «Experimental farm named after Decemberists», the Institute of Swine Production and the Agroindustrial Production of NAAS in Poltava region, which were obtained in 2015–2017. The number of pigs, genotyped by each gene under investigation, is presented in Table 1.

The association of genetic markers with productive traits was determined for the pigs of Mirgorod breed (the breeding farm of SI «Experimental farm named after Decemberists»), using the following indices: age of reaching the bodyweight of 100 kg (days), thickness of backfat, measured at the level of vertebrae 6–7 (mm) at the bodyweight of 100 kg, average daily gain (g) during the feeding period, length of a semi-carcass (cm) and Eye Muscle Area (sq.cm.). DNA from blood samples was extracted using Chelex 100 reagent [12].

DNA-typing using PCR-RFLP by gene *ESR1* (genetic marker *ESR1/PvuII*) was performed according to [13], by gene *RYR1* (g.1843C > T SNP) [14], *PRLR* (*PRLR/Alu I*, SNP c.1789 G > A) [15], *GH* (*GH/BsuRI*) [16], *IGF2* (*IGF2/NciI* (*BcnI*)) [17], *GHRH* (*GHRH/AluI*) [18], *CTSB* (g. 72 A > C SNP) [19], *CTSL* (g.143C > T SNP) i *CTSS* (g.171G > A SNP) [20], *CTSF* (SNP g. 22 G > C) [21], *MC4R* (c.1426 G > A) [22], *LEP* (SNP g.2845 A > T) and *LEP* (SNP g.3996 T > C) [23], *LEP* (SNP g.3469 T > C) [24], *LEPR*

(SNP g.2856C > T) [25], *MUC4* (SNP g.1849G > C) [26] and *FUT1* (SNP g.307 G > A) [27]. DNA-typing by gene *KPL2* was performed using the method, presented in the work [28].

DNA amplification with PCR was conducted using a programmed thermostat TERTSIK-2 (DNA-Technologies, Russian Federation) according to the instructions of DNA-polymerase manufacturer (Thermo Fisher Scientific). The restriction analysis using relevant endonucleases was conducted according to the manufacturer's instructions (Thermo Fisher Scientific). PCR products were separated using 2 % agarose gel-electrophoresis in 1 × Tris-borate electrode buffer (TBE) for 2 h at the current of 50 mA in the

electrophoretic chamber (Cleaver Scientific Ltd, UK). DNA of plasmid *pUC19*, hydrolyzed by endonuclease *Msp I* (Thermo Fisher Scientific), was used as a marker of molecular mass. After electrophoresis, the gel was stained with the solution of ethidium bromide (10 mg/cc) and the electrophoresis results were documented with the digital camera using the transilluminator [29].

DNA-typing of animals by gene *CTSK* (SNP g.15G>A) was conducted by the method of allele discrimination (*Taq Man* – analysis), and by SNP c.1987C>T of gene *LEPR* – by *HRM* (High Resolution Melting) using CFX96 Touch™ Real Time PCR Detection System (Bio-Rad, Laboratories Inc., USA) [30].

Table 1. Frequencies of alleles and genetic variability in the population of pigs of Mirgorod breed by 22 genes of quantitative trait loci

Gene (polymorphism)	<i>n</i>	Frequencies of alleles		PIC	H _e
RYRI (SNP g.1843C>T)	68	<i>C</i> = 0.96	<i>T</i> = 0.04	0.074	0.077
KPL2 (presence/absence ins.)	50	<i>M</i> = 1.00	<i>m</i> = 0.00	0.000	0.000
ESR1 (ESR1/PvuII)	28	<i>A</i> = 0.62	<i>B</i> = 0.38	0.360	0.471
PRLR (SNP c.1789 G>A)	61	<i>A</i> = 0.27	<i>B</i> = 0.73	0.317	0.394
GH (GH/BsuRI)	37	<i>A</i> = 0.89	<i>B</i> = 0.11	0.177	0.196
IGF2 (IGF2/NciI (BcnI))	17	<i>A</i> = 0.74	<i>B</i> = 0.26	0.311	0.385
CTSB (SNP g.72 A>C)	20	<i>A</i> = 0.93	<i>C</i> = 0.07	0.122	0.130
CTSK (SNP g.15G>A)	43	<i>A</i> = 0.16	<i>G</i> = 0.84	0.233	0.269
CTSS (SNP g.171G>A)	20	<i>A</i> = 0.23	<i>G</i> = 0.77	0.292	0.354
CTSL (SNP g.143C>T)	50	<i>C</i> = 0.99	<i>T</i> = 0.01	0.019	0.020
CTSF (SNP g. 22 G>C)	50	<i>C</i> = 0.59	<i>G</i> = 0.41	0.367	0.484
MC4R (SNP c.1426 G>A)	38	<i>A</i> = 0.75	<i>G</i> = 0.25	0.305	0.375
GHRH (GHRH/Alu1)	14	<i>A</i> = 0.32	<i>B</i> = 0.68	0.341	0.435
LEPR (SNP c.1987C>T)	36	<i>C</i> = 0.56	<i>T</i> = 0.44	0.371	0.493
LEPR (SNP c.232T>A)*	50	<i>A</i> = 0.86	<i>T</i> = 0.14	0.212	0.241
LEP (SNP g.2845 A>T)	50	<i>A</i> = 0.64	<i>T</i> = 0.36	0.355	0.461
LEP (SNP g.3469 T>C)	50	<i>T</i> = 0.79	<i>C</i> = 0.21	0.277	0.332
LEP (SNP g.3996 T>C)	50	<i>T</i> = 0.65	<i>C</i> = 0.35	0.352	0.455
FUT1 (SNP g. 307 G >A)	49	<i>G</i> = 0.79	<i>A</i> = 0.21	0.277	0.332
MUC4 (SNP g.1849G>C)	50	<i>G</i> = 0.80	<i>C</i> = 0.20	0.269	0.320
OPNin6 (ins/del of SINE element)2	50	<i>A</i> = 0.14	<i>B</i> = 0.86	0.212	0.241
ACTN1 (ACTN/BstE II) * *	50	<i>G</i> = 0.68	<i>A</i> = 0.32	0.341	0.435
FSHβ (FSHβ/HaeIII) * *	50	<i>A</i> = 0.82	<i>B</i> = 0.18	0.252	0.295
PLIN (SNP g.4119A>G) * *	50	<i>A</i> = 0.58	<i>G</i> = 0.42	0.369	0.487
PLIN (SNP g.7966T>C) * *	50	<i>C</i> = 0.63	<i>T</i> = 0.37	0.358	0.466
Share of polymorphic loci				88.0	
Average expected heterozygosity				0.326	
Average number of alleles per locus				1.96	
Effective number of alleles				1.48	

Note. *n* – number of animals; PIC – polymorphism informational content of a genetic marker; * – data about the frequencies of alleles, obtained in the work of N.K. Sarantseva et al. [32]; ** – data about the frequencies of alleles, obtained in the work of V.Yu. Nora et al. [33]; H_e – expected heterozygosity

GENALEX 6 program was used to estimate population characteristics [31]. The analysis of associations between genotypes and indices of productive traits of pigs was conducted using single-factor disperse analysis (ANOVA, analysis of variance) in Excel 2007.

RESULTS AND DISCUSSION

Table 1 presents the frequencies of alleles of 22 genes of quantitative trait loci, determined both by the results of our own genotyping of pedigree stock of Mirgorod pig breed, and in the investigations of other authors, the works of which have been referenced.

The analysis of allele frequencies demonstrated some specificities of the Mirgorod breed in comparison with local and transborder breeds of different performance lines [34].

For instance, by ryanodine receptor 1 gene (*RYRI*), the frequency of minor allele g.1843T, associated with the hereditary drawback of pigs – enhanced stress sensitivity, the critical manifestation of which is malignant hyperthermia [14], was 0.04. However, the phenotypic manifestation of the mentioned allele was not registered in the Mirgorod breed herd. At the same time, allele g.1843T is absent in local breeds – Ukrainian White Steppe and Ukrainian Spotted Steppe, and in meat breeds it occurs with the frequency from 0.1 to 1.00.

Estrogen receptor 1 gene (*ESR1*), traditionally viewed as a genetic marker of reproductive qualities of sows (*PvuII*-polymorphism of 3rd intron of the gene) [35–37] in the Mirgorod breed, was remarkable for polymorphism similar to most other domestic and foreign breeds. However, according to our data, contrary to the Large White breed, the «desired» allele *ESR1* was not associated with multiple pregnancy of sows in the Mirgorod breed.

Enhancing reproductive traits in pig breeding involves the use of genetic markers, based on polymorphism of prolactin receptor gene (*PRLR*, *AluI*-polymorphism, SNP c.1789 G>A) [38] and follicle-stimulating hormone beta-subunit gene, *HaeIII*-polymorphism) [39]. According to our data and the results, obtained in the work [40], the mentioned genes are notable for polymorphism in the Mirgorod breed; no specificities in terms of allele frequencies and differences from other breeds were determined.

According to the data, presented in [40], secreted phosphoprotein 1 gene (*OPN* or *SPP1*) [41], which has registered impact on such reproductive traits of pigs as a number of piglets at birth and motility of spermia in

boars, was notable for not a high level of polymorphism (indel in SINE element) at the frequency of a minor allele A – 0.136. The distribution of allele frequencies in the population of the Mirgorod breed had slight differences from that for Large White, Large Black, Poltava Meat and Landrace breeds.

As for the growth hormone gene (*GH*), involved in the control of meat and finishing qualities of pigs [42], as of 2017, both alleles of the gene (*BsuRI*-polymorphism) were found in the population of the Mirgorod breed, whereas our previous studies of this breed did not determine allele B of this marker. Allele B was absent in local breeds of Ukrainian White Steppe and Ukrainian Spotted Steppe, and in a number of other breeds, produced in Ukraine, this gene occurred with different frequencies of alternative alleles.

In terms of a physiological function of encoded proteins and localization of some QTLs in regions, cathepsin genes are referred to the candidate genes, involved in the control of meat productivity traits, meat quality and carcass structure [19–21, 43–46]. As for allele frequencies of cathepsin genes S, F, and B, the Mirgorod breed does not have any significant differences from other domestic and foreign breeds, for which relevant population studies were conducted [47]. At the same time, its specificity was a relatively high frequency of the minor allele A by SNP of gene *CTSK* g.15G>A ($q = 0.16$) and practically no polymorphism of gene *CTSL* by SNP g.143C>T ($q = 0.01$).

Leptin genes (*LEP*) and leptin receptor genes (*LEPR*) are also candidate genes to QTL, as they play an important role in regulating lipid exchange [48, 49]. In the Mirgorod breed, allele frequencies by genetic markers *LEPR* (SNP c.1987C>T) and *LEPR* (SNP c.232T>A), associated with the features of finishing and meat traits, had no significant differences from the frequencies of these alleles in the populations of other breeds, in particular, in Large White, according to the data, obtained in [32]. Among genetic markers of leptin gene SNP g.2845 A>T, SNP g.3469 T>C and SNP g.3996 T>C, the Mirgorod breed differed from other breeds in terms of allele frequency only by the latter. For instance, Yorkshire and Large White breed of Ukrainian selection had only one allele (*LEP* g.3996 C) [23], whereas the Mirgorod breed demonstrated the prevalence of allele *LEP* g.3996 T.

Melanocortin 4 receptor gene (*MC4R*) (SNP c.1426 G>A)) and growth hormone-releasing hormone gene (*GHRH*)(*AluI*-polymorphism), impacting the finishing traits of pigs and fat deposition [50, 51] in the Mir-

gorod breed were characterized by a considerable level of polymorphism by the corresponding genetic markers. However, these genes were also polymorphic in a number of other breeds, bred in Ukraine [52, 30].

Genes, impacting the processes of fat deposition, include perilipin gene (*PLIN*) [53]. According to [40] polymorphisms SNP g.4119A > G and SNP g.7966T > C occurred in the Mirgorod breed with rather high frequencies of alternative alleles (Table 1), which were close to the frequencies of these alleles in the Large White breed.

Actinin alpha 1 gene (*ACTN1*), associated with the development of muscle tissue, meat quality and reproductive traits, was represented in the population of Mirgorod breed by two alleles G and A (BstE II – polymorphism) with the prevalence of the former [40], which did not distinguish it from other breeds, investigated by this locus [54].

The mutant allele of calpastatin 2 gene (*KPL2/m*), which differs from the normal («wild» type), by the insertion of transposon in the area of intron 30, and is known to be responsible for immotile, short-tail sperm defect (ISTS) in boars, was not found in the population of the Mirgorod breed [55]. This defect is manifested in animals, homozygous by the mutant allele, and in Finnish Yorkshire, mainly. It has recently been demonstrated that the carriers of this allele may be pigs of other breeds [56].

Mucin 4 gene (*MUC4*) encodes mucin 4 – glycoprotein, playing a relevant role in protecting intestinal epithelium from pathogenic microorganisms, in particular, from adhesive strains of *Escherichia coli*. A point replacement of g.1849 G > C in intron 7 of the gene conditions the existence of two alleles, including g.1849 G, associated with the resistance of pigs to *Escherichia coli* bacteria, causing colibacteriosis [57]. One of the specificities of the Mirgorod pig breed was found to be high frequency of allele g.1849 G of mucin 4 gene. Contrary to a number of other breeds, it was 0.8, whereas, for instance, in the populations of the Large White the allele g.1849 G frequency was in the range of 0.16–0.67 [58].

Fucosyltransferase 1 gene (*FUT1*), encoding α -fucosyltransferase 1, is also associated with the resistance of pigs to diseases, caused by coliform bacilli. The animals, homozygous by recessive allele A of gene *FUT1* (SNP g.307 G>A), are notable for resistance to colibacteriosis, the mutation of gene G→A in position 307 b.p. is «desired» in pigs. In the Mirgorod breed, the

frequency of allele g.307 A was at the level, close to the frequency of this allele in Duroc breed (0.21 and 0.28, respectively), and in different populations of the Large White it was in the range of 0.06–0.43 [27]. Thus, no specific features in terms of the distribution of gene *FUT1* alleles were found in the Mirgorod breed.

In general, the analysis of 25 SNPs of 22 genes demonstrated a high level of genetic variability in the population of pigs of Mirgorod breed. Only three out of all the investigated genes were found to be monomorphic (the accepted criterion of polymorphism was the frequency of a minor allele ≥ 0.05). Therefore, the percentage of polymorphic loci was 88.0 % with the consideration of several polymorphisms, present in one gene. To compare the Mirgorod breed against other breeds (in particular, Large White of different selection, Large Black, Landrace, Ukrainian White Steppe, Ukrainian Spotted Steppe, and Poltava Meat), the percentage of polymorphic loci was calculated separately for 12 genes of QTL from the investigated ones, namely, the ones, previously used by us for genotyping in subpopulations of these breeds (*RYRI*, *ESRI*, *PRLR*, *GH*, *IGF2*, *CTSB*, *CTSK*, *CTSS*, *CTSL*, *MC4R*, *GHRH*, *LEPR*) [47, 30]. In this case, the percentage of polymorphic loci in the Mirgorod breed was 83.3 %, whereas, for instance, in different subpopulations of Large White (of English selection and intrabreed types of ULW-1 and ULW-3) it fluctuated in the range of 58.3–66.7 %, in the population of Large Black – 75.0 %. The percentage of polymorphic loci was also lower (75 % and 50 %, respectively) in the local breeds, Ukrainian White Steppe and Ukrainian Spotted Steppe, which are «closed» and in which the selection is conducted similar to that for the Mirgorod breed, mostly without the involvement of genetic material of other breeds.

Noteworthy is the fact that the average expected heterozygosity H_e (Nei's genetic diversity) was at a relatively high level – 0.326, which is another feature of high genetic variability in the Mirgorod breed. The latter is also confirmed by the average number of alleles per locus – 1.96 and the effective number of alleles – 1.48.

In addition, a high level of QTL gene polymorphism in the population of the Mirgorod breed, as one of the factors of genetic variability, is indicated by the indices of PIC, estimated for each genetic marker, used for typing. For instance, PIC values of 17 out of 25 genetic markers in the investigated population were at the level, exceeding 0.250, which corresponded to their high informative value, sufficient to conduct associative studies in marker-associated selection [59].

The abovementioned data demonstrate the absence of genetic erosion and contraction of genetic variability in the population of pigs of Mirgorod breed. This is in good agreement with the conclusions, obtained via the analysis of its genetic structure by immunological [60] and ISSR [61] markers and microsatellite loci [10]. This also corresponds to the conclusion, made by the results of the analysis of genealogy of intrabreed structures about the possibility of avoiding affinity breeding and preserving the gene fund [62].

More detailed genetic characterization of the Mirgorod breed involved the analysis of the association of specific genetic markers with the main productive traits of the pigs of Mirgorod breed (Table 2). The animals of the experimental group under investigation were estimated by five parameters of the productive traits and genotypes by genetic markers *IGF2/NciI* (*BcnI*), *RYRI* (SNP g.1843C > T), *MC4R* (SNP c.1426 G > A), *LEP* (SNP g.2845 A > T), *GH/BsuRI*, *CTSF* (SNP g. 22 G > C). By the first two markers, all the animals in the experimental group had similar genotypes, *IGF2* AA and *RYRI* g.1843CC, respectively. As for the rest of genetic

markers, pigs were divided into groups in accordance to the genotypes, among which the groups of animals with genotypes *MC4R* c.1426 GG and *GH* BB were absent. Unfortunately, the latter is explained by a small sampling of the animals for associative analysis.

It was determined that there were statistically significant associations of the genetic marker *MC4R* SNP c.1426 G>A with such indices as the age of gaining 100 kg, the thickness of backfat and the Eye Muscle Area. We also determined the associations between the growth hormone gene polymorphisms (*GH/BsuRI*) and the age of gaining 100 kg and cathepsin gene F (*CTSF* SNP g. 22 G > C) with the Eye Muscle Area. There was a tendency, observed for statistically significant differences between the groups of pigs with different genotypes by leptin gene (SNP g.2845 A > T) by thickness of backfat ($p = 0.09$). For comparison, there was no association between the genetic marker *LEP* SNP g.2845 A > T and thickness of backfat in the Large White.

The results regarding the association of SNP and the productive traits for Mirgorod breed with the consider-

Table 2. The associations of genetic markers and productive traits of the Mirgorod breed pigs

Genotype	Productive traits				
	Age of gaining 100 kg (days)	Thickness of backfat (mm)	Average daily gain (g)	Length of semi-carcass (cm)	Eye Muscle Area (sq.cm.)
<i>MC4R</i> SNP c.1426 G > A					
<i>AA</i>	210.3 ± 4.61	32.8 ± 1.02	575.0 ± 22.47	93.9 ± 1.57	31.7 ± 1.01
<i>AG</i>	196.5 ± 3.12	29.3 ± 0.86	621.1 ± 40.22	94.5 ± 0.64	34.3 ± 0.64
<i>P</i>	0.03	0.02	0.33	0.76	0.05
<i>LEP</i> SNP g.2845 A > T					
<i>AA</i>	195.6 ± 3.81	29.4 ± 0.91	579.5 ± 31.50	93.3 ± 1.55	33.0 ± 0.85
<i>TA</i>	209.3 ± 4.88	32.6 ± 1.10	606.3 ± 30.98	93.4 ± 0.63	32.2 ± 0.75
<i>TT</i>	200.1 ± 2.97	29.4 ± 1.40	602.7 ± 76.92	97.0 ± 3.60	34.9 ± 0.77
<i>P</i>	0.13	0.09	0.89	0.30	0.12
<i>GH/BsuRI</i>					
<i>AA</i>	199.7 ± 2.33	31.1 ± 0.94	581.3 ± 22.54	94.1 ± 1.32	33.2 ± 0.69
<i>AB</i>	205.5 ± 6.23	31.2 ± 1.54	633.7 ± 56.25	94.7 ± 1.60	33.2 ± 0.83
<i>P</i>	0.03	0.78	0.57	0.88	0.53
<i>CTSF</i> SNP g. 22 G > C					
<i>CC</i>	201.3 ± 4.30	31.9 ± 1.22	571.5 ± 21.15	94.9 ± 1.52	33.9 ± 0.63
<i>GC</i>	214.6 ± 8.87	29.4 ± 0.74	610.5 ± 36.97	92.8 ± 1.17	30.8 ± 1.23
<i>GG</i>	200.1 ± 3.01	30.2 ± 0.99	643.7 ± 69.26	93.4 ± 1.42	32.4 ± 0.81
<i>P</i>	0.25	0.44	0.42	0.67	0.04

Note. The data are presented as LSMeans ± SEM; the values of *P* in italics correspond to statistically significant differences between the groups.

ation of a small sampling of the experimental animals may be viewed as preliminary data only and should have been validated using a larger number of animals. However, the elimination of the purebred herd and a limited number of remaining Mirgorod breed pigs exclude this possibility. However, the associations, determined in our study, give some ideas about the search for genetic markers of productive traits in pigs of the restored population of the Mirgorod breed for marker-associated selection.

Therefore, the results of the studies on genetic structure of the Mirgorod breed by a number of SNPs and associative analysis demonstrate its specificities and differences from other breeds, bred in Ukraine. First of all, these specificities refer to the general high level of genetic variability, determined by the results of our analysis of SNPs. This is in agreement with the statements of other authors about the Mirgorod breed, whose genetic structure was studied using other classes of genetic markers. The abovementioned proves the common thesis about local breeds, preserving higher level of genetic variability compared to the commercial breeds. The latter are subject to considerable breeding impact, aimed at the maximal manifestation of productive traits, which may result in some contraction of allelic diversity in their populations [63].

It should also be noted that the Mirgorod breed is remarkable for the absence of the mutant allele *KPL2/m*, conditioning genetic anomaly of ISTS, and the recessive allele *RYR1* g.1843T, associated with stress sensitivity of animals, is present with very low frequency (0.04). The latter is the consequence of «new incoming blood» of Pietren – the breed, which was not included into the breeds-founders of the Mirgorod breed. It should be considered that the specificity of the breed is rather a high frequency of the minor allele *CTSK* g.15A ($q = 0.16$) and, thus, rather a high level of informative value of the genetic marker *CTSK* (SNP g.15G > A) (PIC = 0.233). Noteworthy is the polymorphism of gene *LEP* in the Mirgorod breed by the genetic marker of SNP g.3996 T > C, which distinguishes it from the Large White.

CONCLUSIONS

The associations between some genetic markers and productive traits have been determined in the herd of the Mirgorod breed. On the one hand, these associations may result from the immediate impact of polymorphisms of quantitative trait nucleotides (QTN) on their manifestation. However, they may be considered as a specific case of population dynamics process, due

to which there are changes in the frequencies of alleles in the sampling not only by the candidate genes of QTL, but also by a number of others, functionally related or linked to them. The latter is relevant in the context of preserving genetic structure and variability of breeds to prevent the loss of their breed specificity, which is especially urgent for numerically insignificant local breeds. In both cases, in our opinion, it is reasonable to check the presence of the very associations, determined in the original breed, during the breeding process using genetic markers in the restored population of the Mirgorod breed.

It is reasonable to carry out the restoration of the gene pool of the Mirgorod pig breed, taking into account the results regarding the SNPs of the studied genes and their associations with the productive traits, presented in this article. The selection of animals for restoration should be carried out after typing the young animals, giving preference to pigs with genotypes *c.1426 MC4R*^{GA}, *MC4R*^{AA}, *g. 22 CTSE*^{CC}, *g.2845 LEP*^{TT}.

Генетична характеристика миргородської породи свиней, отримана шляхом аналізу одонуклеотидних поліморфізм генів

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Мета. Визначити генетичні характеристики миргородської породи свиней за 25 SNPs 22 генів та провести асоціативний аналіз генів *MC4R* (SNP *c.1426 G>A*), *LEP* (SNP *g.2845 A>T*), *GH* (*BsuRI*-поліморфізм), *CTSE* (SNP *g. 22 G>C*) з ознаками продуктивності тварин.
Методи. Для досліджень були використані зразки крові племінних свиней миргородської породи племзавода ДП «ДГ ім. Декабристів» Полтавська область. ДНК-типуння виконували методами ПЛР-ПДРФ та *TaqMan*.
Результати. Встановлено особливості породи щодо частот алелів генів, високого рівня генетичної мінливості ($He = 0,326$) та алельного різноманіття (середня кількість алелів на локус – 1,96). Алель *KPL2/m*, який спричиняє генетичну аномалію ISTS у свиней миргородської породи відсутній, а рецесивний алель *RYR1* g.1843T, відповідальний за стресчутливість свиней, зустрічається з низькою частотою (0,04). На відміну від інших порід спостерігали відносно високу частоту мінорного алеля *CTSK* g.15A (0,16) і поліморфізм гену *LEP* (SNP *g.3996 T > C*) ($He = 0,455$). Встановлено статистично значущі асоціації поліморфізмів: *MC4R* (SNP *c.1426 G>A*) з

віком досягнення тваринами маси 100 кг, товщиною шпиків та площею «м'язового вічка», *GH/BsuRI* з віком досягнення 100 кг, і *CTSF* (SNP g. 22 G>C) з площею «м'язового вічка». Спостерігали тенденцію щодо статистично значущих відмінностей між групами свиней з різними генотипами *LEP* (SNP g.2845 A > T) за товщиною шпиків ($p = 0,09$). **Висновки.** Відновлення генофонду миргородської породи доцільно проводити з урахуванням даних *SNPs* досліджених генів та їх асоціацій з продуктивними ознаками. Для відтворення породи віддавати перевагу свиням із генотипами с.1426 *MC4R GA*, *MC4R AA*, g. 22 *CTSF CC*, g.2845 *LEP TT*.

Ключові слова: миргородська порода свиней, генетична характеристика, *QTL*, *SNP*.

Генетическая характеристика миргородской породы свиней, полученная путем анализа однонуклеотидных полиморфизмов генов

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Цель. Определить генетические характеристики миргородской породы свиней по 25 *SNPs* 22 генов и провести ассоциативный анализ генов *MC4R* (SNP с.1426 G>A), *LEP* (SNP g.2845 A>T), *GH* (*BsuRI*-полиморфизм), *CTSF* (SNP g. 22 G>C) с признаками продуктивности животных. **Методы.** Для исследований были использованы образцы крови племенных свиней миргородской породы племзавода ГП «Опытное хозяйство им. Декабристов» Полтавская область. ДНК-типирование выполняли методами ПЦР-ПДРФ и TaqMan. **Результаты.** Установлены особенности породы по частотам аллелей генов, высокого уровня генетической изменчивости ($H_e = 0,326$) и аллельного разнообразия (среднее количество аллелей на локус – 1,96). Аллель *KPL2/m*, который вызывает генетическую аномалию *ISTS*, у свиней миргородской породы отсутствует, а рецессивный аллель *RYR1* g.1843T, ответственный за стресс-чувствительность свиней, встречается с низкой частотой (0,04). В отличие от других пород наблюдалась относительно высокая частота минорного аллеля *CTSK* g.15A (0,16) и полиморфизм гена *LEP* (SNP g.3996 T > C) ($H_e = 0,455$). Установлены статистически значимые ассоциации полиморфизмов: *MC4R* (SNP с.1426 G > A) с возрастом достижения животными массы 100 кг, толщиной шпика и площадью «мышечного глазка», *GH/BsuRI* с возрастом достижения 100 кг, и *CTSF* (SNP g.22G>C) с площадью «мышечного глазка». Наблюдалась тенденция статистически значимых различий

между группами свиней с различными генотипами *LEP* (SNP g.2845A > T) по толщине шпика ($p = 0,09$). **Выводы.** Восстановление генофонда миргородской породы, целесообразно проводить с учетом данных *SNPs* исследованных генов и их ассоциаций с продуктивными признакам. Для воспроизведения породы отдавать предпочтение свиньям с генотипами с.1426 *MC4R GA*, *MC4R AA*, g. 22 *CTSF CC*, g.2845 *LEP TT*.

Ключевые слова: миргородская порода свиней, генетическая характеристика, *QTL*, *SNP*.

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