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Original Paper

Study of Polymorphism of the *MC4R* Locus in Pigs of the Large White Breed

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DNA typing was performed by the PCR-RFLP method at the *loci* of the *MC4R* gene The herd of pigs was divided according to origin. Polymorphism of the studied gene is established. The higher frequency of AA and AG genotypes was in animals of French breeding, and the homozygous GG genotype was in crossbred animals. Accordingly, allele A had a higher frequency in French breeding animals and allele B – in crossbred animals. According to the studied gene, genetic balance is absent only in crossbred animals. A high coefficient of genetic similarity was established between French and Belgian breeding animals. Most of the existing breeders have a heterozygous genotype.

Keywords: genetic similarity, allele, genotype, pigs, gene

1 Introduction

The use of modern achievements in genetics makes it possible to significantly speed up the breeding process in various branches of animal husbandry. Thanks to the study of the polymorphism of individual genes, it is possible to effectively improve individual performance indicators of animals (Malikova et al., 2024; Malikova et al., 2024). Melanocortin receptor-4 (*MC4R*) is one of the promising genes that can be used to improve pigs' economic and valuable traits. Scientists believe that its role in regulating energy homeostasis is significant. It has been proven that it directly affects the signs of fattening and meat productivity of pigs (Kim et al., 2020; Li et al., 2006; Dvořakova et al., 2011; Kim et al., 2006). The melanocortin-4 receptor (*MC4R*) has also been shown to be important in the control of energy balance, which mediates the effects of leptin (LEP) on nutrient uptake and energy expenditure (Benoit, 2000). *MC4R* affects metabolism, body weight, and food preference and controls energy homeostasis in mammals (Kim et al., 2000; Kim et al., 2004).

A large body of research suggests that a biological feature of *MC4R* is controlling eating behavior. Thus, a mutation was found in it that causes pigs to eat more (about 10%), grow faster (6–8%), and gain more live weight (6–10%) (Salajpal et al., 2007; Pang et al., 2006; Szyndler-Nkdza et al., 2010).

The missense mutation Asp298Asn in the amino acid sequence of this receptor in mammals promotes obesity (Houston et al., 2004). The mutant allele causes a feeling of hunger, increased appetite, and, as a result, higher rates of increase in the live weight of animals. (Chen et al., 2004).

The *MC4R* gene is located on pig chromosome 1 in the region q22–q27 (Kim et al., 2000). Its alleles are

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characterized by dominant inheritance and the absence of other phenotypic pathology except excessive salinity. Most studies have found a clear correlation between different allelic states of the *MC4R* gene and feeding behavior in various breeds of pigs. Certain contradictions arose primarily with the heterozygosity of experimental animals (Barb et al., 2004) when the decisive role of this gene in regulating food intake was in question.

It was established that allele A determines fast growth and a significant thickness of lard, and allele G is responsible for growth efficiency and a large percentage of lean meat. Homozygous pigs with the AA genotype reach market weight three days faster than pigs homozygous for the G allele (GG), but pigs with the GG genotype have 8% less fat and have a higher feed conversion (Kim et al., 2006).

1.1 The Objective of Our Study

To investigate the peculiarities of the genetic structure of the *MC4R* gene of a herd of pigs of a large white breed of various origins.

2 Material and Methods

The research was conducted in 2024 on a herd of large white pigs – 178 sows and 9 breeding boars. the pigs belonged to the State Enterprise of the Institute of Agriculture of the Northeast of the National academy of agrarian sciences of Ukraine. The biomaterial (bristle) of pigs was used for genetic research. Genetic studies were carried out in the scientific genetics laboratory of the Institute of Pig Breeding and Agro-Industrial Production of the National academy of agrarian sciences of Ukraine.

Sows were divided into three groups by origin: French breeding (n = 105), Belgian breeding (n = 38), and sows combining 1/2 French breeding × 1/2 Belgian breed (n = 35). Knuri, respectively, French selection – (n = 8), Belgian – (n = 1). DNA was isolated from biomaterial samples using Chelex-100 ion exchange resin (Walsh et al., 1991). DNA typing was performed by the PCR-RFLP method (Glazko, 2001) at the *loci* of the *MC4R* gene (Muñoz et al., 2011) (Table 1).

We used a set of amplification reagents from TAPOTYLY and Helicon for PCR-PDRF analysis. We used Fermentas enzymes for DNA restriction according to the manufacturer's recommendations. Electrophoresis in 8% polyacrylamide gel and 2% agarose gel was used to analyze restriction fragments. For visualization, polyacrylamide gel was stained with ethidium bromide and viewed in ultraviolet light on a transilluminator. Photo documentation was carried out with a Canon digital camera. (To conduct PCR-RFLP analysis, a set of reagents for amplification from TAPOTYLY and Helicon was used. DNA restriction was performed using Fermentas enzymes according to the manufacturer's recommendations. Restraint fragments were analyzed using electrophoresis in an 8% polyacrylamide gel and a 2% agarose gel. Visualization was performed by staining the polyacrylamide gel with ethidium bromide, followed by viewing in ultraviolet light on a transilluminator. Photo documentation was carried out with a Canon digital camera)

The following formula was used to calculate the genotype frequency:

$$P_A = \frac{n_A}{N}$$

where: P – the proportion of individuals with trait A; number of animals with sign A; total sample size (Merkuryeva, 1977)

The frequency of alleles was calculated according to the formula:

$$P(A) = \frac{2N1 + N2}{2n}$$

where: N_1 and N_2 – number of homozygotes and heterozygotes for the studied allele, respectively; n – sample number. In order to assess the statistical reliability of the discrepancy between the distribution of the obtained results the Pearson criterion was used:

$$\chi^2 = \frac{\sum (A-T)^2}{T}$$

where: A – actual number of genotypes; T – theoretical number of genotypes (Ladyka et al., 2023)

Table 1 PCR amplification conditions, PCR-RFLP fragments of gene alleles

Genes	The structure of primers for PCR	PCR*	PCR-RFLP fragments of different alleles
MC4R	F:5′-TACCCTGACCATCTTGATTG-3′ R: 5′-ATAGCAACAGATGATCTCTTT-3′	220/60/2,5	PCR-RFLP (TaqI): allele c.1426 A 220 bp; allele c.1426 G 150 + 70 bp

* PCR product size (n.p.)/annealing temperature (°C)/[$MgCl_2$ (mM)]

The actual (available) heterozygosity was determined by direct calculation using the following formula:

$$H_O = \frac{N2}{n}$$

The expected heterozygosity was determined using the following formula:

$$H_E = 1 - \sum_{i=1}^{n} p_i^2$$

where: p_1, p_2,… p_n – frequency of alleles. The fixation index was calculated using the following formula:

$$F_{is} = \frac{H_E - H_O}{H_E}$$

The level of polymorphism was calculated according to the formula:

$$Na = \frac{1}{Ca}$$

where: *Na* – the level of polymorphism; *Ca* – the homozygosity coefficient (according to Robertson)

The degree of homozygosity was calculated according to the formula:

$$Ca = \sum p_i^2$$

where: *Sa* – the degree of homozygosity; *Ri* – the frequency of allele (*i*)

The coefficient of genetic similarity was calculated according to the formula:

$$r = \frac{\sum x \cdot y}{\sqrt{\sum x^2 \cdot y^2}}$$

where: *x* and *y* – the frequencies of one allele in different groups of animals

The coefficient of possible variability (according to Robertson) was calculated according to the formula:

$$V = \left(\frac{(1 - Ca)}{1 - \frac{1}{n}} \right) \cdot 100$$

where: *n* – the number of studied animals (Merkuryeva, 1977)

All statistical calculations were performed using generally accepted methods (Kovalenko et al., 2010).

3 Results and Discussion

As a result of our research, three possible genotypes for the AA, AG, and GG genes were identified among the studied pigs of various origins (Fig. 1).

A significant difference was found in the frequency of possible genotypes between animals of different origins. A higher frequency of the homozygous AA

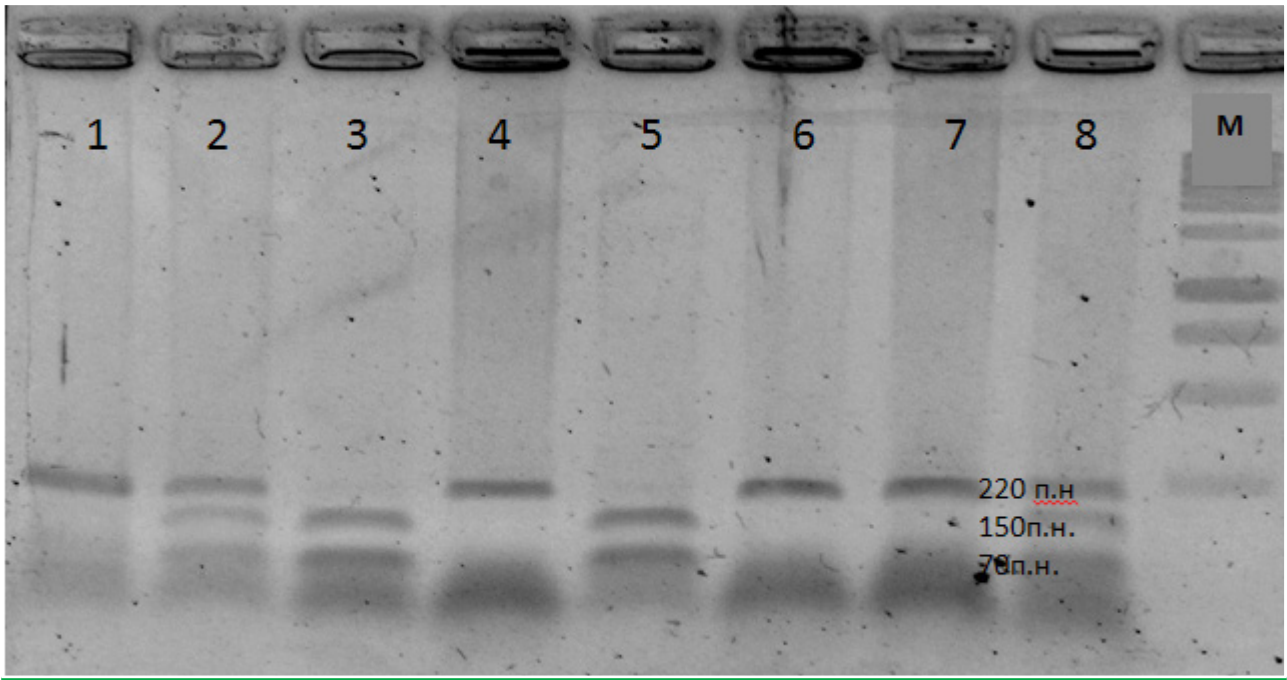

Figure 1 Electrophoresis in 2% polyacrylamide gel of *MC4R* gene restrictions
Track: 1, 4, 6, 7 genotype AA; 2, 8 genotype AG; 3, 5 genotype GG *MC4R* – gene

genotype is associated with a higher fat content and a higher growth rate, characteristic of French breeding animals. A higher share of the homozygous GG genotype associated with lower fat content and better feed conversion is characteristic of crossbred animals 1/2 French selection X 1/2 Belgian selection. A higher proportion of heterozygous AG genotypes is observed in French and Belgian breeding animals. Among animals of different origins, there is an excess of the frequency of homozygous genotypes over the theoretical value of the frequency and a lack of heterozygous genotypes (Table 1).

According to the existing difference in the frequency of genotypes, a difference in the frequencies of alleles was also established. Animals of French breeding are characterized by an almost balanced allele frequency with a slight advantage of allele A. On the contrary, animals of Belgian selection are characterized by a slight advantage of the frequency of allele G. In crossbred animals, 1/2 French selection X 1/2 Belgian selection, the frequency of the G allele is three times higher than the frequency of the A allele. alleles in animals of different origins were statistically significant (reliability criterion tA and tB >3) (Table 2).

Analyzing the indicators characterizing the genetic structure of the *MC4R* gene, we can note that the genetic balance is disturbed only in crossbred animals 1/2 French x 1/2 Belgian crossbreeds; this confirms the value of the χ^2 criterion (8.74), which corresponds to the probability level of $P<0.05$.

The empirical ratio of the number of heterozygotes to the number of homozygotes (K) in animals of different origins was less than one (from 0.22 to 0.72). Accordingly, the actual homozygosity in all animals of all selection options was lower than the theoretical heterozygosity.

This is confirmed by the positive value of the Fisher index (Fis) and the negative value of the heterozygosity test (TG).

The negative value of the kurtosis coefficients (D) also indicates a particular deficiency of heterozygous genotypes.

The degree of homozygosity (Ca) and the level of polymorphism (Na) were almost the same in French and Belgian breeding animals. In crossbred animals, the first indicator was slightly higher, and the level of polymorphism was correspondingly lower. Regarding the degree of realization of possible variability, no difference was found between French and Belgian breeding animals. However, crossbred animals had a slightly lower value of this indicator (Table 4).

Our results indicate a certain similarity between French and Belgian pigs in the genetic structure of the *MC4R*

Table 2 Study of frequencies of genotypes of the *MC4R* gene locus

Distribution	Distribution of genotypes					
	AA		AG		GG	
	n	frequency	n	frequency	n	frequency
French selection						
Factual	39	0.37	44	0.42	22	0.21
Expected	35.7	0.34	51.5	0.49	17.8	0.17
Belgian selection						
Factual	8	0.22	15	0.39	15	0.39
Expected	6.5	0.17	18.6	0.49	12.9	0.34
Crossbreeds 1/2 French selection X 1/2 Belgian selection						
Factual	5	0.13	6	0.18a**в*	24	0.69 a**в**
Expected	1.8	0.05	11.9	0.34	21.3	0.61

p – level of significance according to Fisher's test: * P <0,05; ** P <0.01; a – to the French selection; b – to the Belgian selection

Table 3 Study of frequencies *MC4R* of alleles of the gene locus

Animal origin	Alleles, un.	
	A ±mA	G ±mG
French selection	0.581 ±0.034	0.419 ±0.034
Belgian Selection	0.417 ±0.058	0.583 ±0.058
Crossbreeds 1/2 French selection X 1/2 Belgian selection	0.218 ±0.047	0.782 ±0.047

Table 4 Analysis of the genetic structure of the *MC4R* gene

Animal origin	Distribution	Indicators of heterozygosity							*V*	*Na*	χ_2	*D*	Proportion of homozygotes, %
		Heterozygote	Homozygous	*K*	*H*	F_{is}	*TG*	*Ca*					
French selection	factual	44	61	0.72	0.419	0.139	-0.228	0.513	49.2	1.95	2.04	-0.139	58.1
	expect.	51	54	0.94	0.487								
Belgian selection	factual	14	22	0.64	0.389	0.200	-0.310	0.514	49.9	1.95	1.44	-0.200	61.1
	expect.	18	18	1.00	0.486								
1/2 French X 1/2 Belgian crossbreeds	factual	7	32	0.22	0.179	0.473	-0.298	0.659	34.9	1.51	8.74	-0.474	82.1
	expect.	13	26	0.50	0.314								

Table 5 Coefficient of genetic similarity between animals of different origins

Origin	Origin		
	French selection	Belgian selection	1/2 French X 1/2 Belgian crossbreeds
French selection	–	0.948	0.784
Belgian selection	0.948	–	0.657
1/2 French X 1/2 Belgian crossbreeds	0.784	0.657	–

Table 6 Genotypes of available breeding boars in the herd according to the *MC4R* gene

Origin	Genotypes		
	AA	AG	GG
France	1	6	1
Belgium	–	1	–

gene. This is also confirmed by the coefficient of genetic similarity we calculated. Its value among animals of the specified selections reaches almost one. On the contrary, the value of the coefficient between animals of French and Belgian selections and crossbred animals 1/2 French X 1/2 Belgian crossbreeds has a lower value, which may indicate a lower genetic similarity between them (Table 5).

Animals of all three possible genotypes represent breeding boars of French breeding present on the farm. Most of them (75%) are animals with a heterozygous genotype. If necessary, we can increase the frequency of the required allele (A or G) among French breeding animals. One representative of breeders of Belgian breeding has a heterozygous genotype AG, which allows maintaining the genetic structure of pigs of Belgian breeding at the existing level (Table 6).

Therefore, the use of available boars allows obtaining all possible genotypes for the studied gene (AA, AG, GG).

Our results partially coincide with the results of other researchers. Thus, in animals of French breeding, according to the results of our research, the frequency of genotypes (AA – 0.37; AG – 0.42; GG – 0.21) almost corresponded to the frequency of genotypes obtained by other researchers (AA – 0.35; AG – 0, 50; GG – 0.15) (Likhach et al., 2021). However, the frequencies of the genotypes of the Belgian selection and hybrid genotypes differed significantly, respectively (AA – 0.22; AG – 0.49; GG – 0.34) and (AA – 0.13; AG – 0.18; GG – 0.69). The presence of polymorphism of the *MC4R* gene was also reported by other scientists (Budakva et al., 2021).

4 Conclusions

According to the study results, a polymorphism of the *MC4R* gene was found in pigs of the large white breed, regardless of origin. The presence of three genotypes (AA, AG, GG) with different frequencies in French and Belgian breeding animals and their crosses were revealed. The highest frequency of homozygous

AA genotype characterizes animals of French breeding. A higher frequency of the homozygous GG genotype characterizes crossbred animals. The heterozygous genotype was more common in French and Belgian breeding animals.

According to the genetic structure, animals of different origins have their characteristics. It was established that the genetic balance was disturbed only in crossbreeds 1/2 French X 1/2 Belgian crossbreeds. The actual heterozygosity in pigs, regardless of origin, was lower than the theoretical one, which was confirmed by the positive value of the Fisher index and the negative values of the heterozygosity test and kurtosis coefficients.

The evaluation of the genotype of the available breeding boars testified to the possibility of directed breeding with pigs of French breeding.

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