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# POLYMORPHISM OF RYRI, ESR, MC4R AND LEP GENES IN PIG MICRO-POPULATION OF LARGE WHITE BREED OF UKRAINIAN SELECTION

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*Genetic and population analysis of RYRI, MC4R, LEP and ESR gene polymorphism for Large White breed of pigs of Ukrainian selection was performed. Traditional breeding methods, based on the assessment of animals for their own productivity and the productivity of offspring, do not always provide the expected genetic progress. Advances in molecular genetics in recent years, the development of DNA marker systems of various classes give breeders a new powerful tool for analyzing the genotypes of animals, which allows for selection based on objective genetic information. Particular attention is paid to the so-called SNP markers, the nature of which is associated with single nucleotide polymorphism of DNA in the structural or regulatory parts of genes involved in the control of important economic and biological characteristics of animals. Such genes include RYRI, ESR, MC4R and LEP. DNA typing was performed in the genetics laboratory of the Institute of Pig Breeding and Agro-Industrial Production. For the study, DNA was used, which was isolated from the bristles of pigs of Large White breed in LLC "Druzhba-Kaznacheiivka", including124 heads. DNA typing was performed using PCR-RFLP. In the micropopulation of the Large White breed there is a certain polymorphism in the genes ESR, MC4R and LEP. All experimental animals in the RYRI gene are monomorphic and had the genotype p.1843CC, a variant of the mutant allele was absent. The presence of a significant amount of allele B-0.61, (61 %) and high frequencies of genotypes BB and AB (0.42 and 0.38), which are associated with high rates of sow multiplicity, allow to select animals by the desired genotypes to increase reproductive rate in sows of the studied micro-population. Quite a high level of homozygous TT genotypes with a frequency of 0.85 leptin gene affected the possibility of its using in marker selection of the animals. A sufficient level of the index of polymorphic information content of PIC by genes ESR and MC4R indicates that marker selection of Large White breed in LLC "Druzhba-Kaznacheiivka"is possible.*

***Key words:*** *Large White breed of pigs, marker selection, leptin gene, estrogen receptor gene, ryanodinreceptor gene, melanocortin-4 receptor, single nucleotide polymorphism.*

# ПОЛІМОРФІЗМ ГЕНІВ RYRI, ESR, MC4R ТА LEP У МІКРОПОПУЛЯЦІЇ СВИНЕЙ ВЕЛИКОЇ БІЛОЇ ПОРОДИ УКРАЇНСКОЇ СЕЛЕКЦІЇ

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*У роботі проведено генетико-популяційний аналіз поліморфізму генів RYRI, MC4R, LEP та ESR для великої білої породи свиней української селекції. Традиційні методи селекції, що засновані на оцінці тварин за власною продуктивністю та продуктивністю нащадків, не завжди забезпечують очікуваний генетичний прогрес. Досягнення молекулярної генетики останніх років, розробка систем ДНК-маркерів різних класів дають селекціонерам новий потужний інструмент для аналізу генотипів тварин, що дає змогу проводити відбір і підбір, зважаючи на об'єктивну генетичну інформацію. Особливо велика увага приділяється так званим SNP-маркерами, природа яких повязана з однонуклеотидним поліморфізмом ДНК у структурній або регуляторній частинах генів, що беруть участь у контролі важливих господарських і біологічних ознак тварин. До таких генів належать RYRI, ESR, MC4R та LEP. ДНК-типування проводили в лабораторії генетики Інституту свинарства і АПВ. Для дослідження використано ДНК, яку виділено зі щетини свиней великої білої породи тварин агрофірмування СГ ТОВ «Дружба-Казначеївка» в кількості 124 голови. ДНК-типування проводили з використанням техніки ПЛР-ПДРФ. У мікропопуляції великої білої породи існує певний поліморфізм за генами ESR, MC4R та LEP. Усі дослідні тварини за геном RYRI мономорфні та мали генотип с.1843СС, варіант мутантного алеля був відсутній. Присутність значної частки алелю В-0,61, (61 %) та високих частот генотипів ВВ та АВ (0,42 та 0,38), які мають асоціацію з високими показниками багатоплідня свиноматок, дозволяють відбирати тварин за бажаними генотипами для підвищення багатоплідня у свиноматок у досліджуваній мікропопуляції. Досить високий рівень гомозиготних генотипів ТТ з частотою 0,85 гену лептину вплинув на можливість його використання в маркерній селекції на цій вибірці тварин. Достатній рівень індексу поліморфного інформаційного змісту PIC за генами ESR та MC4R свідчить, що проведення маркерної селекції на вибірці свиней великої білої породи СГ ТОВ «Дружба-Казначеївка» є можливим.*

**Ключові слова:** *велика біла порода свиней, маркерна селекція, ген лептину, ген естрагенового рецептора, ріанодинрецепторний ген, рецептор меланокортина-4, однонуклеотидний поліморфізм.*

## Introduction

Traditional selection methods, based on the assessment of animals for their own productivity and the productivity of offspring, do not always provide the expected genetic progress. Advances in molecular genetics in recent years, the development of DNA marker systems of various classes give breeders a new powerful tool for analyzing the genotypes of animals, which allows for selection and selection based on objective genetic information.

Particular attention is paid to the so-called SNP markers, the nature of which is associated with single nucleotide polymorphism of DNA in the structural or regulatory parts of genes involved in the control of important economic and biological characteristics of animals. Such genes include RYRI, ESR, MC4R and LEP.

Ryanodinreceptor gene (RYRI) is associated with pigs stress-resistant, the negative manifestation of which is the development of malignant hyperthermic syndrome [1]. Fujii, J. et al. [2] identified a mutation at position 1843 C>T in the RYRI gene (chromosome 6 (6p12-q22), which in the homozygous state causes increased stress in animals. Such pigs are characterized by high carcass meat content, but low meat quality and poor reproductive function.

One of the most important features in pig breeding is reproductive, in particular, the reproductive rate of sows. A number of genes are involved in the control of this trait, but the closest association with fertility has been established for the estrogen receptor (ESR) gene [3]. It is known allelic variants associated with single nucleotide polymorphism at the restriction sites of endonucleases Pvu II, Ava I and MspA1I [4, 5]. In a number of studies for different breeds and lines of pigs, it was shown that sows with the BB genotype according to the Pvu II polymorphic restriction site are superior to animals with AB and AA genotypes in the number of newborn piglets, from 0.6 [6] to 3.58 [7] piglets, on the nest.

There are a number of genes that are directly involved in the formation of fattening and meat qualities of pigs. In particular, it is the melanocortin-4 (MC4R) receptor gene [8]. The melanocortin-4 MC4R receptor is

directly involved in adipose tissue metabolism as one of the components of a complex system of eating behavior. The MC4R gene is located on pig chromosome 1 in region (SSC1) q22-q27 [9]. It was found that in some pig populations, the single-nucleotide polymorphism MC4R Asp298Asn of this gene is significantly associated with fattening and meat qualities, in particular with the thickness of lard [10].

The next gene that affects pig meat, fattening and meat quality is the leptin gene (LEP). Leptin, in addition to participating in lipid metabolism, is involved in the regulation of the immune response and control of reproductive function, affects the growth and formation of bone tissue. The pig leptin gene is located on chromosome 18, its length reaches almost 17 thousand nucleotide pairs, consists of 3 exons and 2 nitrons [11]. The key role of leptin regulation of lipid metabolism at the body level allows to identify clear associations between different allelic variants of the leptin gene and lipid metabolism parameters associated with the accumulation of fat in the subcutaneous tissue and between internal organs [12]. A number of single-nucleotide polymorphisms have been identified in the porcine leptin gene, localized both in its exonic and intron regions and in untranslated regions (UTRs) adjacent to the 5'- and 3'-genes.

The SNP g.2845 A>T polymorphism localized in the 2nd intron will be studied.

It is obvious that the typing of animals by these genes can provide useful information for the selection of pigs with a favorable genotype for the development of traits. However, information on the polymorphism of these genes in pig breeds bred in Ukraine and breeding work is practically absent. It is not known whether marker selection for these genes on an intrabreed basis is possible. However, if a polymorphism is found for these genes, it will provide a basis for breeding work aimed at consolidating certain productive traits in the study population.

*The aim* of the study was to genetic and population analysis of RYRI, MC4R, LEP and ESR gene polymorphism for a large white breed of pigs of Ukrainian selection was performed.

## Materials and methods of research

All procedures related to animals were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [13].

DNA typing was performed in the genetics laboratory of the Institute of Pig Breeding and APV. For the study, DNA was used, which was isolated from the bristles of pigs of Large White in LLC "Druzhba-Kaznacheiivka, having 124 heads. Isolation of DNA from biomaterial samples was performed using ion exchange resin Chelex-100 [14].

DNA typing was performed using PCR-RFP technique [15] at the loci of genes MC4R [16], Leptin (LEP) [17], RYR1 [1] and ESR [18]. The structure of the primers, PCR conditions and the corresponding restriction fragments of alternative alleles for each locus (table 1).

***1. Conditions of PCR amplification, PCR-RFLP fragments of gene alleles.***

| Genes | The structure of primers for PCR | PCR [1] | PCR-RFLP fragments of different alleles |
|---|---|---|---|
| *RYR1* | F:5'- GTGCTGGATGTCCTGTGTTCCCT -3'<br>R:5' - CTGGTGACATAGTTGATGAGGTTTG -3' | 134/68/2,0 | PCR-RFLP (*Hha I*): allele g. 1843T (n) 134 bp; allele g. 1843C (N) 84 + 50 p.n. |
| *MC4R* | F:5 '-TACCCTGACCATCTTGATTG-3'<br>R: 5 '-ATAGCAACAGATGATCTCTTT-3' | 220/60/2,5 | PCR-RFLP (*Taq*I): allele c.1426 A 220 bp; allele c.1426 G 150 + 70 bp |
| *Leptin(LEP)* | F:5 '- TTGGCGAGCCTGGAGCAGT -3'<br>R: 5 '- GCAGCCTCCATCCCTAAGTGGG -3' | 242/55/2,0 | PCR-RFLP (*XbalI*): allele g.2845A, 242 p.n.; allele c. g.2845T, 170 + 72 p.n. |
| *ESR* | F:5 '- CCTGTTTTTACAGTGACTTTTACAGAG -3'<br>R: 5 '- CACTTCGAGGGTCAGTCCAATTAG -3' | 120/56/2,0 | PCR-RFLP (*Pvu II*): allele A.120 p.n.; allele B. 65 + 55 p.n. |

*PCR product size (bp) / annealing temperature (° C) / [MgCl2 (mM)].*

A set of reagents for amplification by TAPOTILI and Helicon (Russia, Moscow) was used for PCR-RFLP analysis. Restriction of DNA was performed using enzymes from Fermentas (Lithuania, Vilnius) in accordance with the manufacturer's recommendations.

Restriction fragment analysis was performed by 8 % polyacrylamide gel electrophoresis. Visualization was performed by staining the polyacrylamide gel with ethidium bromide, followed by viewing in ultraviolet

light on a transilluminator. Photo documentation was performed with a Canon digital camera.

## Research results and their discussion

All experimental animals were tested for the presence of the p.1843C>T mutation in the ryanodine receptor gene, which is associated with swine sensitivity and meat defects, and had the p.1843CC genotype, the mutant allele variant was absent.

All three variants of gene genotypes (ESR, MC4R and LEP) were identified. Typical electrophoregram of restriction fragments of the corresponding genes (Fig. 1; Fig. 2).

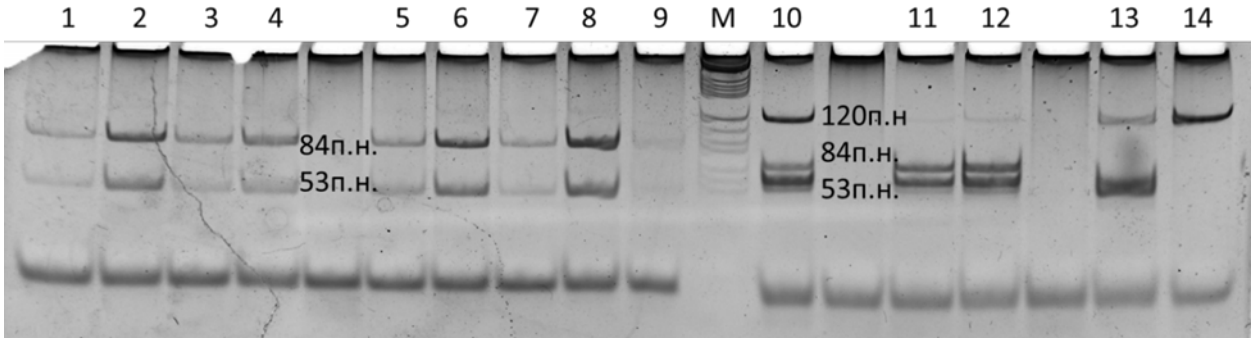


***Fig. 1. Electrophoresis in 8 % polyacrylamide gel restriction of RYR1 and ESR genes. Track:*** *1–9 genotype NN RYR1 – gene, track: 10, 13 genotype AB, track: 11, 12 genotype BV, track: 14 AA, ESR – gene. M is a molecular weight marker pBR322 DNA / BsuRI*

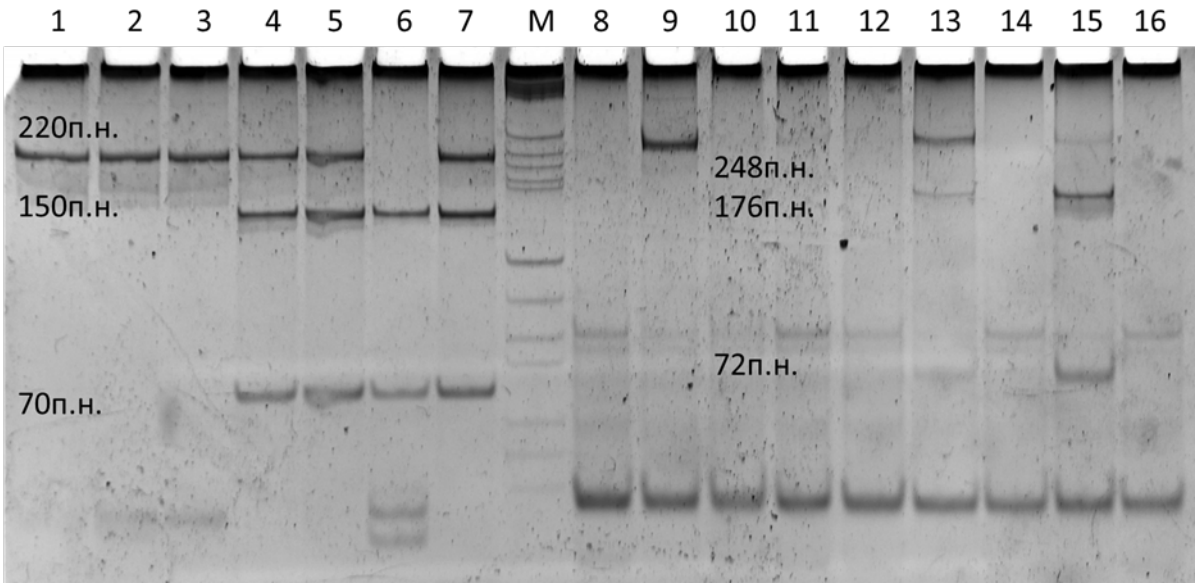


***Fig. 2. Electrophoresis in 8 % polyacrylamide gel restriction MC4R and Leptin (LEP) genes:*** *Track: 1–3 genotype AA, track: 4, 5, 7 genotype AG, track: 6 genotype GG, MC4R gene. Track: 8, 10, 11, 12, 14, 15, 16 genotype TT, track: 9 genotype AA, track: 13 genotype AT, Leptin (LEP) – gene. M – molecular weight marker pBR322 DNA / BsuRI*

At the locus of the ESR gene there is a higher frequency of genotypes BB and AB (0.42 and 0.38, respectively) (Table 2). Sows of Large White breed are characterized by relatively high fertility rate, which is reflected in the presence of a significant proportion of allele B-0.61, (61 %) Fig. 3 and high frequency of genotypes BB and AB, which are associated with high fertility sows.

For the ESR gene, a statistically significant difference between the expected frequencies of genotypes relative to the actual $\chi 2=5.171$ was found. Detection of the shift from equilibrium according to Hardy-Weinberg's law, indicates the effect of artificial or natural selection of animals. The Wright fixation index F=0.204, indicates a slight increase in homozygous animals in the micropopulation.

In the population of Large White pig breed in LLC “Druzhba-Kaznacheiivka”, MC4R gene was also polymorphic, the frequency was dominated by genotypes AA and AG (0.39 and 0.42, respectively). The G

allele of the MC4R gene, which is associated with a smaller thickness of sebum, occurred with a frequency of 0.40. A significant difference between the expected and actual frequency of genotypes was not found $\chi 2$=2,052. The fixation index was insignificant (F=0.129).

***2. Frequency distribution of genotypes in the micropopulation of WB breed pigs***

| s / n | Locus | Frequencies of alleles | Frequencies of alleles | | | $\chi^2$ | F |
|---|---|---|---|---|---|---|---|
| | | | AA | AB/AG/AT | BB/GG/TT | | |
| 1 | *ESR* | A=0,39<br>B=0,61 | 0,20<br>(0,15) | 0,38<br>(0,48) | 0,42<br>(0,37) | 5,171* | 0,204 |
| 2 | *MC4R* | A=0,60<br>G=0,40 | 0,39<br>(0,36) | 0,42<br>(0,48) | 0,19<br>(0,16) | 2,052 | 0,129 |
| 3 | *LEP* | A=0,15<br>T=0,85 | 0,10<br>(0,02) | 0,10<br>(0,26) | 0,80<br>(0,72) | 48,759*** | 0,627 |

*Authenticity criteria:* * – P<0.05; *** – P<0.001.

All three variants of genotypes were detected in the leptin gene, but the predominant share was the TT genotype with a frequency of 0.85 and the T allele with a major frequency of 0.85, table 2. Another alternative allele A and genotypes AA, AT had a minor frequency of 0.15 and 0.10, respectively. A significant significant shift of the Hardy-Weinberg equilibrium between the expected and actual frequency of genotypes $\chi 2$=48,759 was revealed. Which indicates a significant selective pressure on the study population for this gene. The fixation index indicates an excessive number of homozygotes with the TT genotype (F=0.627).

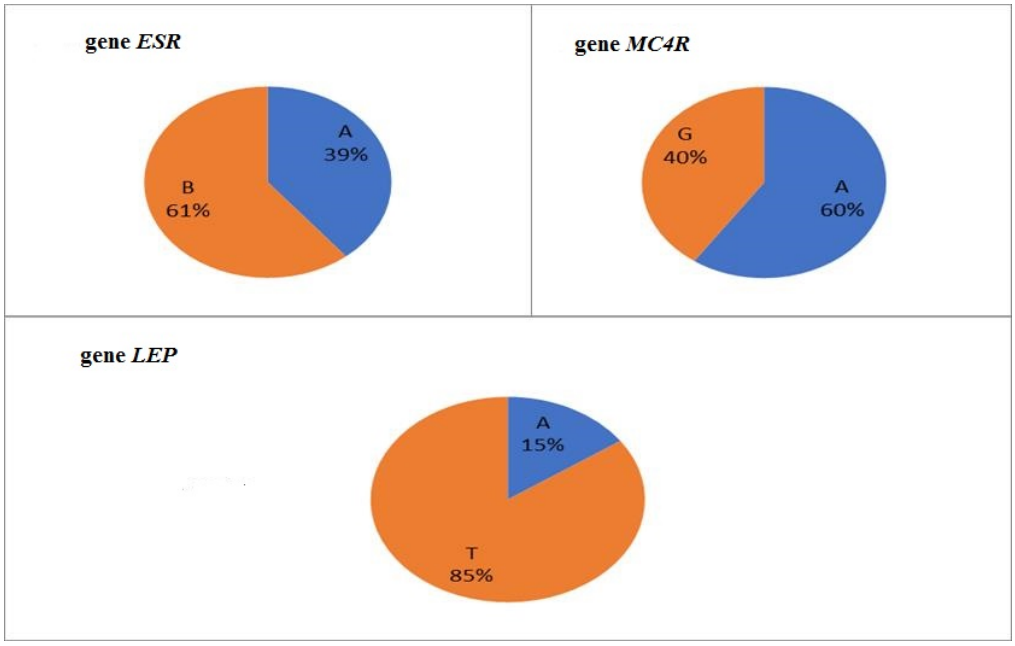


***Fig. 3. Graphical representation of the distribution of the share of alternative alleles by ESR, MC4R and LEP genes***

The level of genetic diversity was determined on the basis of an assessment of the actual (But) and expected (He) heterozygosity. A significant difference between No and Ne was found only by the leptin gene, which indicates the involvement of this gene polymorphism in artificial selection, table. 3.

The index of polymorphic information content PIC (Polymorphism Information Content) of genetic markers LEP SNP g.2845 A>T, ESR and MC4R was calculated by the value of which their level of polymorphism was estimated. The optimal PIC values, which provide the necessary diversity of genotypes to establish their relationships with performance indicators, are in the range from 0.25 to 0.75. According to the ESR and MC4R genes, the PIC level in the large white breed population is optimal (0.36). However, the PIC gene for leptin is low (0.22), (table 3).

*3. Distribution of frequency of gene alleles in populations of pigs of different breeds*

| Locus | Ho | He | PIC |
|---|---|---|---|
| *ESR* | 0,379 | 0,476 | 0,36 |
| *MC4R* | 0,419 | 0,481 | 0,36 |
| *LEP* | 0,097 | 0,259* | 0,22 |

*Criteria of reliability:* * – P <0.05.

## Conclusions

In the micropopulation of Large White breed in "Druzhba-Kaznacheiivka" LLC, there is a certain polymorphism in ESR, MC4R and LEP genes. All experimental animals in RYRI gene are monomorphic and had the genotype p.1843CC, a variant of the mutant allele was absent. The presence of a significant proportion of allele B-0.61, (61 %) and high frequencies of genotypes BB and AB (0.42 and 0.38), which are associated with high rates of multiple sows, allow animals to be selected by the desired genotypes to increase reproductive rate in sows in the studied micro-population. A fairly high level of homozygous TT genotypes with a frequency of 0.85 leptin gene affected the possibility of its using in marker selection in this group of animals. A sufficient level of the index of polymorphic information content of PIC by ESR and MC4R genes indicates that marker selection of Large White pig breed in LLC "Druzhba-Kaznacheiivka" is possible.

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**Бібліографічний опис для цитування:**

*Матіюк В. В., Саєнко А. М., Усенко С. О., Халак В. І.* Поліморфізм генів *RYRI, ESR, MC4R* та *LEP* у мікропопуляції свиней великої білої породи Українской селекції. *Вісник ПДАА*. 2020. № 4. С. 150–156.