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Association of the AspP298Asn polymorphism in the MC4R gene with fattening productivity of immunologically castrated and uncastrated gilts

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PYO: project administration, conceptualization, methodology, funding acquisition, investigation, writing — original draft, review and editing, formal analysis.
PKF: data curation, project administration, methodology, funding acquisition, formal analysis.
BIB: conceptualization, writing — review and editing, formal analysis.

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None to declare.

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During the scientific and experimental study, all international, national and/or institutional principles of animal care and use were followed, in particular: "Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes".

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The study was aimed at establishing the presence of MC4R (c.1426 A>G) gene polymorphism among the population of immunologically castrated and native gilts (Large White × Landrace) × Maxgro. Asp298Asn polymorphism affects the economic rate of growth and fattening productivity of commercial lines of pigs. Since the polymorphism of the MC4R gene correlates with the concentration of androstenone, skatole, and indole, the possible use of polymorphism as a molecular DNA marker MC4R for genetic selection in order to reduce the level of boar odor in gilts meat and fat. This will establish which alleles and genotypes in general will be determined as desirable in the marker dilution of hybrid pigs. With this in mind, the purpose of the study was to determine the effect of the MC4R genotype on fattening performance indicators and correlation with boar odor in hybrid gilts based on population-genetic variability. Genomic DNA was isolated from the ear hair follicle of native gilts (n=42) and epithelial tissue from the ear of immunologically castrated ones (n=52). Genotyping was performed using polymorphism of the lengths of restrictive fragments (RFLP). Genetic-correlation parameters for native and immunologically castrated gilts were evaluated by ADG/AGE30 and ADG/AGE100. However, the polymorphism of the c.1426 A>G gene did not significantly affect the ADG/AGE100 performance of hybrid gilts. The predominance of the frequency of allele A (0.55) is established above the frequency of the allele G (0.45). Analysis of the frequency distribution of genotypes showed the saturation of micropopulation with heterozygotes AG (0.51) with a small proportion of GG carriers (0.19). Immunologically castrated and uncastrated gilts with an $MC4R^{A}$ allele are characterized by a sufficiently high ADG/AGE100 and are probably characterized by a high level of boar-specific odor compared to gilts with lower ADG/AGE100 with an $MC4R^{G}$ allele. According to the data obtained, the desired genotype for an equilibrium of the concentration of boar odor without compromising physiological development, growth rate, and productivity formation is heterozygous offspring with the $MC4R^{AG}$ genotype.

Key words: hybrid gilts, (Large White × Landrace) × Maxgro, native gilts, immunologically castrated gilts, polymorphism, MC4R, Asp298Asn, ADG, AGE30, AGE100, boar smell, genotype, allele, PCR-RFLP analysis

Introduction

The melanocortin-4 receptor (MC4R) gene encodes a membrane-bound receptor protein and is a member of the melanocortin receptor family of genes [6, 13]. One of the popular drawbacks of raising gilts is the presence of boar taint, an off-odor present in heated meat or fat. The main compounds of this odor are androstenone, skatole, and indole [1, 8, 14]. In addition to management strategies of the genetics selection toward lower boar taint incidence could help pig farmers to shift toward raising gilts and boars. An important candidate polymorphism that could serve as the molecular marker is the Asp298Asn polymorphism of the MC4R (c.1426 A>G) gene. The Asp298Asn polymorphism of the melanocortin-4 receptor (c.1426 A>G) in pigs to affect economically important traits such as AGE100. this missense mutation Asp298Asn is associated with increased ADFI, increased ADG, and decreased lean meat percentage [14]. Breeds of pigs with a low lean meat percentage, to have high levels of boar taint and back fat thickness is correlated with androstenone and skatole levels in fat [4, 9, 14]. Therefore, I assume that the Asp298Asn polymorphism of the MC4R gene could serve as a possible marker for boar taint. Allele c.1426 A>G^{A} is characteristic of pigs with lower lean meat percentages and possibly have higher levels of boar taint compared to pigs (allele c.1426 A>G^{G}) with higher lean meat percentages. The interest of the study was the search for associations of the MC4R polymorphism, especially when one genotype is overrepresented in the population of hybrid pigs. Ascertain assess of genetic selection toward lower boar taint levels is possible in the meat and lard of female pigs by using the MC4R marker in commercial pigs, without compromising growth. Scientists assessed the main effects and possible interactions between sex and MC4R genotype in homozygous boars and gilts of the AA and GG genotype. The concentrations of the 3 boar taint compounds androstenone (P=0.044), skatole (P=0.049), and indole (P=0.006) were significantly higher in the fat of boars with the MC4R^{AA} genotype compared to boars with the MC4R^{GG} genotype. An interaction between genotype and sex was evaluated for the parameter ADG (P=0.044): boars with MC4R^{AA} genotype had a significantly higher ADG than MC4R^{GG} boars but there was no significant difference between the gilts. Daily lean meat gain was higher in boars compared to gilts (P=0.051), independent of genotype. Genotype and sex affected the quality parameters of the carcass. It is established that the compound causes the smell of boar, and meat quality was not affected by genotype. The results of the study show that pork of gilts was darker (P=0.014) and less exudative during cooking (P<0.001) and contained more intramuscular fat (P=0.013) [11, 14]. In this regard, the interest was to assess if genetic selection toward lower boar taint levels is possible by using this marker in hybrid gilts and assess the correlation of productivity indicators with the smell of boar.

According to the analyzed scientific sources, world scientists, we concluded that the productivity of pigs is determined by the genotype inherited from the parents. It was analyzed, according to world literary sources, that pigs with the desired genotype under certain conditions are characterized by unexpectedly worse productivity compared to parents carriers of genotypes with lower or higher productivity [3, 7, 10, 12].

The objective of the present study was to examine if there were any associations between variations in the MC4R gene with signs of fattening productivity in a commercial native and immunologically castrated gilts population in Ukraine based on population-genetic variability.

Materials and Methods

The hair follicle and epithelial tissue samples were collected from hybrid pigs of the (Large White × Landrace) of ages (10–12 months) and of different groups: immunologically castrated (n=52) and uncastrated (n=42) kept at the Globyno Pig Complex. Hair follicle samples from the ear of pigs were collected using tweezers or simple plucking by hand. Appropriate measures were observed to avoid contamination of the hair samples. The hair samples were placed in an “Eppendorf”-type test tube, carefully labeled, and stored at a temperature of –20°C in a laboratory of genetics, at the Institute of Pig Breeding and Agro-Industrial Production NAAS. 120–150 µL of 20% *Chelex-100* suspension was added to the contents of the test tubes and incubated for 6 hours at 56°C. After shaking the test tubes on *Vortex*, they were placed in a solid-state thermostat and incubated for 8 min at a temperature of 98°C. DNA solution samples were stored at –20°C [5]. Isolation of DNA from epithelial tissue was carried out using a set for the isolation of nucleic acids DNA-sorb-B of the manufacturer *InterLab Service-Ukraine* LLC [2].

PCR amplification of porcine MC4R gene fragment

The PCR components include 12.5 ng of genomic DNA, 1.25 µl of 1x PCR buffer, 1.0 µl MgCl2, 1.25 µl dNTPs, 0.25 µl of each primer, and 0.4 µl Taq DNA polymerase (*ThermoFisherScientific*™), ultrapure sterile water — 5.0 µL and the final volume of the template DNA 7.6 µL. The total volume of PCR mixture with genomic DNA is 17 µL/1 sample. For the amplification of specific MC4R gene fragments, the following primers by were used: Forward: 5' — *TAC CCT GAC CAT CTT GAT TG*-3', 157.4 µg, 26.0 nmol and Reverse: 5' — *ATA GCA ACA GAT GAT CTC TTT G*-3', 171.8 µg, 25.5 nmol. The components were subjected to the following cycling profile: 3 min at 94°C; 31 cycles at 94°C — 25 min; 64°C — 26 sec; 40 sec at 72°C; and a final 2-minute extension at 72°C in a thermal cycler (*TERTSYK*, DNA Technology). Gel documentation was done after subjecting the amplicons to electrophoresis on 2% agarose gels.

Analyses of SNPs in the MC4R gene

The restriction Fragment Length Polymorphism (RFLP) method was used to detect polymorphisms in the MC4R (rs81219178) gene, using endonuclease TaqI (*ThermoFisherScientific*™) as the restriction enzyme. Enzymatic digestion was performed in a final volume of 15 µL, including 5 µL (~0.1–0.5 µg of DNA) of the PCR product, 0.2 µL of TaqI endonuclease (*ThermoFisherScientific*™), and 2.8 µL Buffer 10x, together with nuclease-free water to reach final volume 7.0 µL. Samples were incubated at 65°C, time — 1 h 50 min for MC4R (TaqI). Electrophoretic separation of DNA fragments was carried out in 8% polyacrylamide gel in 1x TBE buffer, at current strength (5V/cm) gel length. Visualization of restriction products was carried out by dyeing bromide ethidium and viewing on the transilluminator in UV light *(MicroDOC* Gel Documentation Digital camera with UV Transilluminator, *Cleaver Scientific*).

Results and discussion

DNA typing of the studied groups was carried out on the uncastrated (n=42) and immunologically castrated hybrid pigs (n=52) for SNP MC4R (c.1426 A>G).

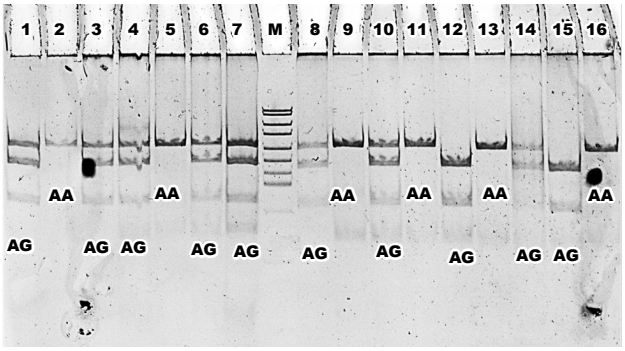

Fig. 1. Electrophoregram of Taq I restriction products of MC4R (c.1426 A>G) locus DNA in 8% PAAG. Molecular marker: *pUC19 DNA/MspI (HpaII) Marker*, 23

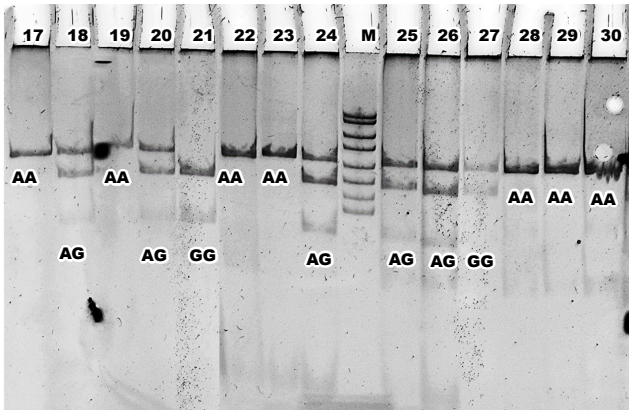

Fig. 2. Electrophoregram of Taq I restriction products of MC4R (c.1426 A>G) locus DNA in 8% PAAG. Molecular marker: *pUC19 DNA/MspI (HpaII) Marker, 23*

DNA typing involves identifying allele gene variants alleles of which are characterized by restriction fragments the size of base pairs (bp). Fragments of the resulting electrophoregram (fig. 1–2): Track — 1, 3, 4, 6, 7, 8, 10, 12, 14, 15, 18, 20, 24–26 with genotype c.1426AG (70 bp.); 2, 5, 9, 11, 13, 16, 17, 19, 22, 23, 28–30 with genotype c.1426AA (220 bp); 21, 27 with genotype c.1426GG (150 bp.). Size of DNA fragments in (bp) major allele MC4R c.1426G (150+70); DNA fragment size in (bp) of the MC4R c.1426A minor allele (220).

According to the results of the electrophoregram (fig. 1–2) it is observed that the studied hybrid piglets are predominantly heterozygous in genotype $MC4R^{AG}$.

Homozygous AA and homozygous GG boars and gilts were produced by crossing hybrid sows (genotype AG; Genetics, Hermitage) with Maxgro terminal boars (also genotype AG).

The analysis of fattening productivity was carried out according to the results of control cultivation up to 100 kg according to the following indicators: the age the birth weight, adjusted 30-day weaning weight (AGE 30), average daily gain (ADG), and growth rate (AGE 100) of gilts; time spent in the rearing group, days; time spent in the fattening group, days (table 1).

Genetic correlations for native gilts by ADG/AGE30 parameters with $MC4R^{AA}$ genotype are +0.67 in relation to $MC4R^{AG}$ genotype and +0.101 in relation to $MC4R^{GG}$ genotype. Immunologically castrated gilts are evaluated according to the same parameters: with $MC4R^{AA}$ genotype are +0.47 in relation to $MC4R^{AG}$ genotype and +0.138 in relation to $MC4R^{GG}$ genotype. In relation to the ADG/AGE100 parameters, the correlation in immunologically castrated gilts with respect to native gilts with genotype $MC4R^{AA}$ is ADG +0.77 and for AGE100 –5 days; for pigs with $MC4R^{AG}$ genotype are ADG +0.85

Table 1. Effect of MC4R genotype on fattening productivity parameters in gilts

Trait	Locus genotype		
I. Native gilts	$MC4R^{AA}$ (n=11)	$MC4R^{AG}$ (n=22)	$MC4R^{GG}$ (n=9)
ADG, g/day	0.748	0.681	0.647
AGE30, day	30	30	30
ADG, g/day	0.940	0.845	0,774
AGE100, day	142	151	159
Time spent in the rearing group, days	44	44	44
Time spent in the fattening group, days	107	107	107
RL, %	1,287	1,262	1,255
II. Immunologically castrated gilts	$MC4R^{AA}$ (n=18)	$MC4R^{AG}$ (n=25)	$MC4R^{GG}$ (n=9)
ADG, g/day	0.797	0.750	0.659
AGE30, day	30	30	30
ADG, g/day	1.017	0.930	0.753
AGE100, day	137	143	154
Time spent in the rearing group, days	44	44	44
Time spent in the fattening group, days	107	107	107
RL, %	1.342	1.312	1.199

Note. ADG — average daily gain; AGE — average daily gain; RL — relative gain.

and for AGE100 –8 days; for MC4R^{GG} genotype are ADG –0.21 and for AGE100 +5 days. Immunologically castrated and uncastrated gilts with the MC4R^{A} allele are characterized by a sufficiently high ADG/AGE100 and could possibly have higher levels of boar taint compared to gilts with lower ADG/AGE100 (MC4R^{G} allele).

Genotyping of micropopulation of hybrid gilts (Large White × Landrace) × Maxgro SPE "Globinsky pig complex" LLC showed that the MC4R (c.1426A>G) locus is polymorphic, the level of which turned out to be sufficient to be able to search for the connection of individual genotypes with performance indicators and for associative research. This is indicated by the value of the calculated index of the polymorphic information content of the locus (0.37) (table 2). The predominance of frequency of allele A (0.55) over allele G (0.45) was established.

Analysis of the distribution of frequencies of genotypes showed the saturation of micropopulation with heterozygotes AG (0.51) with a small proportion of GG carriers (0.19). Animals with the homozygous genotype AA (0.30) accounted for almost a third of the sample analyzed. The resulting spectra frequency genotypes indicate that animals behind this locus are not subject to both targeted and situational selection in this farm, and therefore the herd is not consolidated according to the marker under study, which creates favorable opportunities for marker-auxiliary selection according to MC4R (c.1426A>G). No probable difference was found in the distribution of actual frequencies genotypes from the theoretically possible χ2 method according to Hardy-Weinberg. There is a slight advantage of the actual heterozygosity value (0.509) over the expected (0.495).

To test the working hypothesis about the existence of a significant effect of individual genotypes of the locus c.1426A>G on the formation of fattening characteristics of gilts (Large White × Landrace) × Maxgro dispersion analysis of the obtained experimental results was applied (table 3).

For the sample of pigs (Large White × Landrace) × Maxgro, a probable association of genotypes according to the studied polymorphism of the melanocortin receptor gene with an indicator of average daily gain (P≤0.05) is shown, and the breeding index of fattening qualities (P≤0.01), and the parameters of the influence of the genetic factor on the studied trait were 32.14%, 67.69%, and 8.81%. The lowest ADG score was observed in homozygous pigs on the G allele, while the highest average daily gains were characterized by pigs of heterozygous genotypes. By ranking the selection index of fattening qualities, in which the indicators of average daily weight gain are laid, the group of carrier animals of the AA genotype had a significant advantage over each of the other groups. No statistically significant effect of MC4R (c.1426A>G) on age gain of 100 kg and ADG in the studied micropopulation of pigs (Large White × Landrace) × Maxgro was found.

An experiment was designed to study the effect of MC4R genotype, on the parameters of fattening productivity and boar taint levels in commercial female pigs. The data obtained will form the basis for the development of a methodology for assessing the projected efficacy of SNP MC4R in the population of commercial pigs.

The studied herd of native and immunologically castrated gilts was successfully genotyped for the c.892A>G (p. Asp298Asn) single nucleotide polymorphism (SNP) by using TaqI PCR-RFLP methods. This SNP was significantly associated with (P<0.00017***), (P<0.017**).

The results indicate that genetic selection against boar taint is possible using the MC4R marker. Perhaps this will lead also result in lower feed intake and ADG and, consequently, better carcass quality.

Table 2. The gene frequency of MC4R (c.1426 A>G) in the transboundary breed of gilts (Large White × Landrace) × Maxgro

Genotypes			Gene frequency		Ho	He	PIC	χ^2	Fis
AA	GG	AG	A	G					
0.30/0.30	0.19/0.20	0.51/0.49	0.55	0.45	0.509	0.495	0.37	0.092	–0.029

Note. Ho — actual heterozygosity; He — expected heterozygosity; χ^2 — deviations between empirical and theoretical frequencies of genotypes relative to the Hardy–Weinberg law; PIC (polymorphic information content) — index of the polymorphic information content of the locus; F_{is} — Wright fixation index.

Table 3. Association of individual genotypes of the MC4R (c.1426A>G) locus with the productive performance of gilts

Performance indicators	X±Sx	X±Sx	X±Sx	Ð^2,%	P
	GG	AG	AA		
Age of reaching live weight of 100 kg, days	156.5±5,04	146,96±2.51	139.5±2.91	32.14	0.07
Average daily gain, g	763.5±10.68	887.5±7.43	978.5±7.26	67.69	0.00017***
Breeding index of fattening qualities	96.84±2,16	96.95±2,44	104.84±1,85	8.81	0.017**

Note. Ð^2 — impact strength indicator; P — significance indicator; *** — P≤0,01, ** — P≤0,01, * — P≤0,05 by the criterion of Fisher's significance; X — average for intra-breed type; ±Sx — arithmetic mean error.

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Асоціація поліморфізму Asp298Asn гена MC4R з відгодівельною продуктивністю в імунологічно кастрованих та нативних свинок

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Дослідження було спрямоване на встановлення наявності поліморфізму гена MC4R (c.1426 A>G) серед популяції імунологічно кастрованих та нативних підсвинків (велика біла × ландрас) × Maxgro. Поліморфізм Asp298Asn впливає на економічний показник швидкості росту та відгодівельну продуктивність комерційних ліній свиней. Оскільки поліморфізм гена MC4R корелює з концентрацією андростенону, скатолу та індолу, то можливе використання поліморфізму як молекулярного ДНК-маркера для генетичного відбору з метою зниження рівня запаху кнура у м'ясі та салі свинок дозволить встановити, які алелі та генотипи загалом будуть визначені як бажані у маркерному розведенні гібридних свиней. З огляду на це, метою проведеного дослідження було визначити вплив генотипу MC4R на показники відгодівельної продуктивності та кореляцію із запахом кнура у гібридних підсвинків на основі популяційно-генетичної мінливості. Геномну ДНК виділили з волосяного фолікула вуха нативних (n=42) та епітеліальної тканини з вуха імунологічно кастрованих підсвинків (n=52). Генотипування проводили за допомогою поліморфізму довжин рестриктних фрагментів (ПЛР). Генетико-кореляційні параметри для нативних та імунологічно кастрованих підсвинків оцінювали за показниками ADG/AGE30 та ADG/AGE100. Проте поліморфізм гена c.1426 A>G не вплинув суттєво на показники ADG/AGE100 гібридних підсвинків. Встановлено переважання частоти алеля A (0,55) над частотою алеля G (0,45). Аналіз розподілу частот генотипів показав насиченість мікропопуляції гетерозиготами AG (0,51) з невеликою часткою носіїв GG (0,19). Імунологічно кастровані та некастровані підсвинки з алелем $MC4R^A$ характеризуються достатньо високим ADG/AGE100 і, ймовірно, мають високий рівень специфічного запаху кнура порівняно з підсвинками з нижчим ADG/AGE100 з алелем $MC4R^G$. Згідно з отриманими даними, бажаним генотипом для рівноваги концентрації запаху кнура без шкоди фізіологічного розвитку, швидкості росту та формування продуктивності є гетерозиготне потомство з генотипом $MC4R^{AG}$.

Ключові слова: гібридні свинки, (велика біла × ландрас) × Maxgro, нативні підсвинки, імунологічно кастровані підсвинки, поліморфізм, MC4R, Asp298Asn, ADG, AGE30, AGE100, запах кнура, генотип, алель, аналіз ПЛР-ПДРФ

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