

## Effect of polymorphisms in intron 1 of the swine *POU1F1* gene on growth and reproductive traits

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**Abstract:** The most prospective and actual trend in farm animal breeding is studying gene polymorphisms affecting the productive traits. The objective of this investigation was to study the effect of polymorphisms in intron 1 of the swine *POU1F1* gene on number of days to 100 kg (days to 100 kg), length of body (LB), backfat thickness (BF), and number of piglets born alive (NBA). Research was conducted on purebred pigs of Landrace (n = 80) and Duroc (n = 100) and crossbred pigs (Landrace × Large White) (n = 192). Insertions/deletions in intron 1 of the *POU1F1* gene were defined by the PCR method. In Landrace the frequency of genotypes AA, AB, and BB was 80.0%, 20.0%, and 0.0%; in crossbred pigs it was 63.0%, 29.0%, and 8.0%; and in Duroc it was 100.0%, 0.0%, and 0.0%, respectively. Significant effects of polymorphisms in intron 1 of the *POU1F1* gene have been found in Landrace on days to 100 kg and LB and in crossbred pigs on LB and BF. The effect of the *POU1F1* gene on NBA was not defined in our population. The obtained results show the possibility of using polymorphism in the 1st intron of the *POU1F1* gene as a promising marker in breeding programs for improving growth and meat traits.

**Key words:** Pig, *POU1F1*, polymorphism, Landrace, Duroc, crossbred pig, length of body, backfat thickness

### 1. Introduction

One of the most important priorities in ensuring food security in Russia is improving the breeding and productive qualities of farm animals (1,2). The most prospective and actual trend in farm animal breeding is studying gene polymorphisms affecting the productive traits (3,4). Over the last decade the interest of scientists has been focused on genes encoding growth factors, hormones, receptors, transport, and regulatory proteins (5,6).

Transcription factors represent a group of proteins capable of interacting with DNA regions located in the regulatory area of genes, initiating a program of increasing or decreasing transcriptions. The pituitary factor of *POU1F1* transcription (also known as *Pit-1* or *GHF-1*) stimulates the expression of growth hormone (*GH*) genes, prolactin (*PRL*), and thyroid-stimulating hormone (*TSH*) (7). The presence of the *POU1F1* factor is found at the early stage of embryogenesis in somatotrophs. The Prop-1 factor (prophet of *POU1F1*) determines the initial formation of somatotrophs, prolactotrophs, and thyrotrophs, which are differentiated with the *POU1F1* transcriptional activator being involved (8). *POU1F1*-encoding cDNAs have been

cloned in several mammalian species including cattle, pigs, goats, and sheep (9–13). Pigs' *POU1F1* gene is located on chromosome 13. Mutations in the *POU1F1* gene are associated with growth, meat, and reproductive qualities of pigs (14–17). Yu et al. (14) presented *POU1F1/RsaI* polymorphism of intron 4 in Large White pigs and in Large White × Landrace crossbred pigs. Influence of *POU1F1/RsaI* on selection traits of Large White, Landrace, and Duroc pigs of Polish origin was established in subsequent studies (16,17). Polymorphism in intron 3 of the *POU1F1/MspI* gene was originally established by Tuggle et al. (12). Yu et al. (14) discovered its relation with traits of growth, backfat thickness, and weight of piglets at birth. Song et al. (17) reported that the genetic variability in intron 1 of the *POU1F1* gene (insertions or deletions from 313 pairs of the bases) was associated with better growth of piglets and genotype frequencies varied depending on the breed. In their recent studies, Kim et al. (18) showed the relationship of genetic variability in intron 1 of the *POU1F1* gene with backfat thickness, carcass weight, pH, and color values in crossbred pigs (Landrace × Yorkshire × Duroc). The results of these studies demonstrate the impact of

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insertions/deletions in intron 1 of the *POUIF1* gene on the important selection traits of pigs. The aim of this study was to investigate the effect of polymorphism in intron 1 of the swine *POUIF1* gene on the growth and reproductive traits in purebred pigs of Landrace and Duroc and in crossbred pigs (Landrace × Large White).

## 2. Materials and methods

### 2.1. Experiment material

Research was conducted on purebred pigs of Landrace (♀n = 80) and Duroc (♂n = 100) and on crossbred pigs (Landrace × Large White) (♀n = 192) bred in Russia. Pigs of 1 year were selected. All pigs were kept under identical standardized conditions. The productivity of pigs was evaluated according to the following traits: number of days to 100 kg (days to 100 kg), length of body (LB), thickness of backfat (BF), and number of piglets born alive (NBA). BF was determined with a Sono-Grader-Model2 instrument (Renko Corporation, USA). The measurement was carried out on the back central line, 10–11 cm behind the withers that corresponds to the level of the 6th–7th thoracic vertebra. LB was measured with a centimeter tape from the crests to the tail root. NBA was evaluated by the first three litters.

### 2.2. PCR analysis

The analyses of the pigs' genomic DNA were performed in the Molecular Diagnostics and Biotechnology Laboratory of Don State Agrarian University. To carry out the molecular genetic studies, sample tissues of 1 cm<sup>2</sup> were taken from the ear area of the animals. Samples were placed into tubes, which were filled with 96% alcohol and stored in refrigerators at a temperature of –20 °C. DNA was isolated using a kit of reagents, DIAtom DNA Prep 100 (LLC Research and Production Company Genlab, Russia). The insertions/deletions of intron 1 polymorphisms of the *POUIF1* gene were determined by PCR method. Two primers suggested by Kim et al. (18) were used: forward 5' - CAT TCC CAT TCT GCC ATT TG - 3' and reverse 5' - CCT GTT GCT GTG TTT CCC AG - 3'. The PCR amplification (25 µL of final volume) was performed using 20 ng of genomic porcine DNA, 1X PCR buffer (Evrogen, Russia), 100 µM of each dNTP, 10 pmol of each primer, and 2 U of Taq polymerase (Evrogen, Russia). Amplification conditions were as follows: 94 °C for 5 min, then 35 cycles at 94 °C for 30 s, 57 °C for 60 s, and 72 °C for 60 s with the final cycle of 72 °C for 7 min. Fragments were separated in 1.5% agarose gel.

### 2.3. Statistical analysis

The allelic and genotypic frequencies, observed (Ho) and expected (He) heterozygosity, and Hardy–Weinberg equilibrium test results were calculated using PopGene 3.1 software. The effect of the *POUIF1* gene on growth traits was examined using the following model of the R package,

version 3.3.2:  $Y_{ijkl} = \mu + \text{POUIF1 } i + \text{Breed } j + \text{Sex } k + e_{ijk}$ , where  $Y_{ijkl}$  is the observed trait (number of days to 100 kg (days to 100 kg), length of body (LB), or backfat thickness (BF));  $\mu$  is the overall mean; *POUIF1* *i* is the effect of the genotype of *POUIF1* (*i* = AA, AB, BB); *Breed* *j* is the effect of breed (*j* = Landrace, crossbred); *Sex* *k* is the effect of sex (*k* = male, female); and  $e_{ijk}$  is random error.

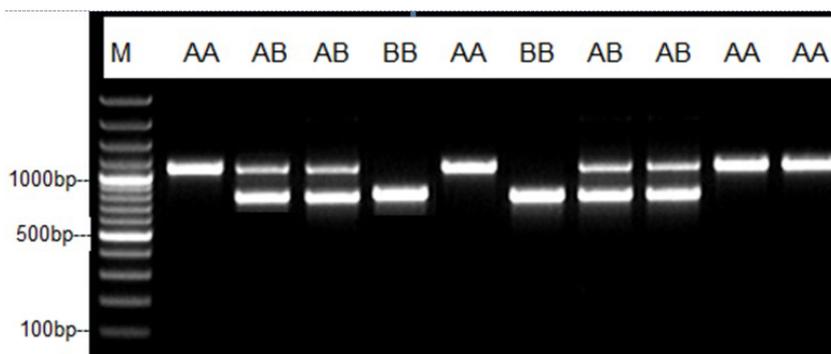
The effect of the gene on reproductive traits was examined using the following model of the R package, version 3.3.2:  $Y_{ijkl} = \mu + \text{POUIF1 } i + \text{Breed } j + P k + e_{ijkl}$ , where  $Y_{ijkl}$  is the observed trait (number of piglets born alive (NBA));  $\mu$  is the overall mean; *POUIF1* *i* is the effect of the genotype of *POUIF1* (*i* = AA, AB, BB); *Breed* *j* is the effect of breed (*j* = Landrace, crossbred); *P* *k* is the effect of parity (*k* = 1 and ≥ 2); and  $e_{ijkl}$  is random error.

The additive and dominant effects were calculated according to the formulas proposed by Folkoner et al. (19):  $a = (AA - BB) / 2$ ;  $d = (AB - (AA + BB) / 2)$ , where *a* is the additive effect, *d* is the dominant effect, AA and BB are the values of the homozygous genotypes, and AB is the value of the heterozygous genotype. In the absence of homozygous genotype BB the effect was calculated as:  $d' = (AB - AA)$ . The significant differences between groups were determined by *t*-test.

## 3. Results

As a result of the PCR analysis of intron 1 of the *POUIF1* gene, we obtained fragments of different lengths that corresponded to genotypes AA (fragment of 1091 bp), BB (fragment of 778 bp), and AB (two fragments of 1091 and 778 bp) (Figure). The frequencies of alleles and genotypes of the gene in different pig breeds are shown in Table 1. The analysis based on the Hardy–Weinberg law resulted in establishing the fact that genetic equilibrium is maintained in the studied population ( $P > 0.05$ ).

The crossbred pigs (Landrace × Large White) demonstrated all three genotypes, AA, AB, and BB. The highest frequency was found for allele A and genotype AA. In Landrace pigs, the homozygous genotype BB was absent and the highest frequency was also found for allele A and genotype AA. The Duroc pigs were not polymorphic. According to the analysis of allele frequencies and genotype, the Duroc pigs were not polymorphic, and so the effect of *POUIF1* genotypes in intron 1 on productivity was evaluated only in Landrace and crossbred pigs. Means of pigs' reproductive and growth traits for different *POUIF1* genotypes are presented in Table 2. The results showed a positive effect of the AB genotype on days to 100 kg ( $d' = 2.15$ ) and LB ( $d' = 124.18$ ) for the Landrace pigs. For crossbred pigs the effect of genotype AB on LB ( $d = 0.73$ ) and genotype BB on BF ( $a = 0.47$ ) were also established. The effect of the *POUIF1* gene on NBA has not been established in our study.



**Figure.** Electropherogram of the PCR analysis of intron 1 of the *POU1F1* gene in crossbred pigs.

M - DNA marker, 1000 bp (SibEnzyme); AA genotype - 1091 bp; BB genotype - 778 bp; AB genotype - 1091 and 778 bp.

**Table 1.** Allele and genotype frequencies of the *POU1F1* gene in different pig breeds.

Breed	Genotype						Allele		$\chi^2$
	AA		AB		BB		A	B	
	n	%	n	%	n	%			
Crossbred	121	63.0	56	29.0	15	8.0	0.77	0.23	2.83
Landrace	64	80.0	16	20.0	0	-	0.90	0.10	1.23
Duroc	100	100.0	0	-	0	-	1.00	-	-

$\chi^2$  - Test value indicates that the different genotypes are in Hardy-Weinberg equilibrium:  $\chi^2_{0.05} = 3.84$ ,  $\chi^2_{0.01} = 6.63$ .

**Table 2.** Means of growth and reproductive traits of pigs of different genotypes of the *POU1F1* gene.

Traits	Genotype, mean $\pm$ SE			a	d
	AA	AB	BB		
Crossbred					
Days to 100 kg	159.17 $\pm$ 1.13	158.35 $\pm$ 2.27	155.25 $\pm$ 2.17	1.95	0.57
LB	121.96 $\pm$ 0.44	123.07 $\pm$ 0.52	121.25 $\pm$ 0.54	0.35	0.73*
BF	13.24 $\pm$ 0.35	12.78 $\pm$ 0.65	12.31 $\pm$ 0.27	0.47*	0.05
NBA	12.64 $\pm$ 0.24	13.08 $\pm$ 0.41	12.93 $\pm$ 0.31	-0.14	0.15
Landrace					
Days to 100 kg	161.06 $\pm$ 1.81	156.75 $\pm$ 1.93	-	d'	
LB	124.18 $\pm$ 1.07	127.25 $\pm$ 2.17	-	2.15*	
BF	11.18 $\pm$ 0.41	10.75 $\pm$ 0.25	-	-1.53**	
NBA	13.02 $\pm$ 0.61	13.20 $\pm$ 0.72	-	0.09	

\* P < 0.05; \*\* P < 0.01.

#### 4. Discussion

Studying polymorphisms of the *POU1F1* gene showed the presence of mutations in various areas and their relation to the productive traits of pigs (14–18). The polymorphism of the *POU1F1* gene in intron 3, determined by the

restriction enzyme *MspI*, is due to the presence of alleles C and D. The homozygous genotype CC was found only in LW and Chinese breeds, and Landrace and Duroc pigs are monomorphic by allele D (14,20). The studies of Yu et al. (14) of several crosses (Meishan  $\times$  Duroc, Meishan  $\times$

Hampshire, Meishan × Landrace, Minzhu × Hampshire, and Minzhu × Landrace) found a very low frequency of genotype CC. However, genotype CC was associated with greater average backfat, first-rib backfat, last-rib backfat, and last lumbar backfat than genotype DD (14).

In our earlier studies (2), polymorphism in Duroc pigs was found in intron 4 of the *POU1F1* gene (*RsaI* restriction enzyme, alleles E and F). The pigs' growth traits (days to 100 kg, average daily gain, length of body, and backfat thickness) were estimated. The results showed a positive effect of genotype EE on length of body, with no significant effect being found for other traits.

Pierzchala et al. (15), in studying polymorphisms of *POU1F1/RsaI* in pigs obtained by mating boars of four breeds (Polish Landrace, Polish Large White, Duroc, or Pietrain) with crossbred sows (Polish Landrace × Polish Large White), found genotype FF to have a significant effect on the meat content of carcasses and the mean daily live weight gain. The effect of genotype EE was established with better fat thickness over the loin and ham-covering fat.

The studies presented in this paper showed the effect of polymorphism in intron 1 of the *POU1F1* gene on days to 100 kg, LB, and BF for Landrace and crossbred pigs. The data obtained showed the presence of a positive effect of genotype AB on LB in Landrace and crossbred pigs. However, the effect of genotype BB on BF was established only for crossbred pigs. Perhaps this is due to the lack of genotype BB in Landrace pigs. Kim et al. (18) studied the effect of genotypes on meat qualities (backfat thickness,

carcass weight, pH, and color values) of crossbred pigs (Landrace × Yorkshire × Duroc). The results did not show any significant differences in BF associated with polymorphism in intron 1 of the *POU1F1* gene. A significant positive effect on carcass weight was found with the AB and BB genotypes (18). In this study, a positive effect of genotype AB on days to 100 kg was determined for Landrace pigs.

The presence or absence effects of polymorphisms of the swine *POU1F1* gene on growth and reproductive traits can be explained by differences in genetic structures and linkage disequilibrium in pig breeds. On the whole, the results of the analysis of polymorphism in intron 1 of the *POU1F1* gene show a positive effect of the allelic variant B (genotypes AB and BB) on the growth and meat traits of pigs.

Our research shows the possibility of using polymorphism in intron 1 of the *POU1F1* gene as a promising marker in breeding programs for improving growth and meat traits in the studied pig population. The obtained results suggest the *POU1F1* gene to be a marker for growth and meat traits and prove the expediency of further research to use them in selection programs.

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