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Polymorphisms in Several Porcine Genes are Associated with Growth Traits

Getmantseva Lyubov, Anatoly Kolosov, Maria Leonova, Siroj Bakoev, Aleksander Klimenko, Vyacheslav Vasilenko and Anastasia Radyuk

Don State Agrarian University, Persianovskiy, Russia

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Corresponding Author:
Getmantseva Lyubov
Head of the laboratory of molecular diagnostics and biotechnology of farm animals, Persianovsky, Rostov region, Russia
Email: ilonaluba@mail.ru

Abstract: The purpose of this study was to confirm the relation between polymorphism of *POU1F1*, *GH*, *PRLR* and *MC4R* genes and economically important traits of the Duroc pigs bred in Russia. Studies were carried out on purebred Duroc breed pigs (male n = 360). All pigs were kept under identical and standard conditions. The traits of analysis: The number of Days to 100-kg (Days to 100 kg), Average Daily Gain (ADG), Length of Body (LB) and Backfat Thickness (BF). The additive and dominance effects of genes were calculated. The signification effects were found for *POU1F1* on LB (a = 0.75); *GH* on Days of 100 kg (a = 2.43), LB (d = 0.71); *PRLR* on Day of 100 kg (d = 1.11) and ADG (d = -29.73); *MC4R* on Days of 100 kg (a = 2.58; d = 2.49) and LD (d = -0.83). Our result showed the influence of *POU1F1*, *GH*, *PRLR* and *MC4R* on the growth trait and perceptivity of their use in breeding programs.

Keywords: Growth Traits, Pig, Gene, Polymorphism, *POU1F1, GH*, *PRLR*, *MC4R*

Introduction

The intensification of the livestock industry demands introduction of new effective methods of evaluating animals. Molecular and genetic analysis techniques based on the polymorphic nature of DNA allow rapid identification of genes controlling the formation of various features of animals as well as their productivity (Fontanesi *et al*., 2012; Ma *et al*., 2014; Rostellato *et al*., 2014).

Transcription factors represent a group of proteins capable to interact with the DNA characteristic sectors located in the regulatory pieces of gene and initiating programs of increasing or decreasing transcription. The main function of transcription factors is to read and interpret the genetic information that allows providing each gene with a unique regulation method in the process of an organism's development (Fan *et al*., 2012). The specific transcription factor *POU1F1* (also known as *Pit-1* or *GHF-1*) effectively stimulates the expression of growth hormone genes (GH), of prolactin (*PRL*) and Thyroid Stimulating Hormone (TSH) in the pituitary gland. The growth hormone and prolactin refer to the family of prolactin-like proteins and effect the growth, anabolic, hyperglycemic, lipolytic and lactogenic activity. The growth hormone has the highest anabolic and growth activity. Te Pas *et al*. (2005) reported that on injecting the growth hormone to swine the growth rate and muscle hypertrophy increased, while the growth of muscle hypertrophy, fat storage and the number and size of fat cells decreased. Growth hormone introduction reduces deposition of lipids, regardless of gender, breed and age (Louveau and Gondret, 2004). At the same time Weber *et al*. (2002) notes that the long-term excess of the growth hormone leads to a disease associated with myopathy which induce muscle hypertrophy and feebleness.

Prolactin affects the reproductive quality, development of mammary glands and lactation. The prolactin receptor which refers to membrane receptors associated with cytoplasmic protein kinases is a hormonal signal conductor for both the prolactin and the growth hormone. Molecular studies in the sectors of the hypothalamus periventricular nucleus have revealed the expression of gene melanocortin-4 receptor (*MC4R*) encoding the second type of neuronal melanocortin receptors. These studies have led to the assumption of melanocortin-4 receptor's participation in the regulation of the hypothalamo-pituitary system. In the

Science Publications

research of mice with a knockout *MC4R* gene (Huzar *et al*., 1997) the impact of this gene on obesity was experimentally proved.

Biological features genes of the transcription pituitary factor (*POU1F1*), the growth hormone (GH), the prolactin receptor (*PRLR*) and the melanocortin receptor -4 (*MC4R*) served as an occasion for revealing association between their polymorphism and growth-weight characteristics. In studies Yu *et al*. (1995) found the relationship of *POU1F1* gene's polymorphism with fattening and meat qualities of pigs. Similar results were obtained in the further studies when the effect of *POU1F1*gene's polymorphism on the growth signs was considered (Piórkowska *et al*., 2013; Kim *et al*., 2014). *POU1F1* is located in chromosome 13 and consists of six exons and five introns. Song *et al*. (2007) reported that genetic variation in the intron 1 (insertions or deletions from 313 pairs of the bases) of the *POU1F1* was connected with young pigs' growth, genotypes frequencies with intron 1 varying according to a breed. Yu *et al*. (1995) presented *POU1F1*/MspI polymorphisms in the intron 1 and *POU1F1*/RsaI in the intron 4 in Large White pigs and in the Large White x Landrace cross. In the subsequent researches of Large White, Landrace, Duroc pigs of the Polish selection the influence of *POU1F1*/RsaI (dbSNP rs80904061) on selection signs of pigs was established (Piórkowska *et al*., 2013).

The *GH* is located in swine chromosome 13 and consists of five exons and four introns. A number of investigations of pigs breeds such as Large White, Landrace, Duroc, Pietrain ones demonstrated a significant effect of the *GH* gene's polymorphism on the growth signs, but the effects of *GH* genotypes are not universal and depend on the pigs' breed, line or cross (Pierzchala *et al*., 2003; Faria *et al*., 2006). Polymorphism of *PRLR*/AluI gene (dbSNP rs45435440) is considered mainly for the effect in terms of reproductive indexes. However, the studies of Alonso *et al*. (2003; Do *et al*., 2012) demonstrated the effect of *PRLR* gene on fattening and meat quality of pigs. In our research of hybrids the influence of *PRLR* gene on meat quality was also found (Mihailov *et al*., 2014). The available literature data show that polymorphism of the *MC4R* in pigs is associated with the growth rate, back fat thickness and feeding (Dvorakova *et al*., 2011; Munoz *et al*., 2011). Our results obtained previously from researches on pigs of Danish Landrace, Canadian Landrace and Commercial Crossbreds breeds showed the effect of *MC4R* gene genotypes on Days to 100 kg, ADG and BF (Klimenko *et al*., 2014). Recent studies of Van den Broeke *et al*. (2015) demonstrated the possibility of using *MC4R* in selection against boar taint, as well as for lower feed intake and ADG and consequently for a better carcass quality.

The purpose of this study was to confirm or disprove the relation between polymorphisms of *POU1F1*, *GH*, *PRLR* and *MC4R* genes and economically important traits of the Duroc pigs bred in Russia.

Materials and Methods

Animals

Studies were carried out on purebred Duroc breed pigs (male n = 360) developed to Breeding Farm «Yubileiny» in Russia. The farm specializes in breeding purebred pigs Landrace, Large White and Duroc. The Landrace and Large White breeding are aimed at improving the reproductive traits and Duroc is on growth and meat (Leonova *et al*., 2015). All pigs were kept under identical and standard conditions.

Studied Traits

The productivity of pigs takes account the following traits: The number of Days to 100-kg (Days to 100 kg), Average Daily Gain (ADG), Length of Body (LB) and Backfat Thickness (BF). All traits were obtained according to the results of growing up to 100 kg.

Genotyping

Extraction, manipulation and subsequent analysis of porcine genomic DNA were performed in the Laboratory of molecular diagnostics and biotechnology Don State Agrarian University. DNA was isolated from blood leukocytes using a kit Diatom DNA Prep100 (Isogene Lab.Ltd.Russia). Specific oligonucleotide primers for the PCR were constructed on base of literature data (Table 1). The PCR amplification (25 μL final volume) was performed using 20 ng of genomic porcine DNA, 1×PCR buffer (Evrogene, Russia), 100 μM each dNTP, 10 pmol each primer and 2 U Taq polymerase (Evrogene. Russia).

Conditions were: for *POU1F1* -94°C for 4 min, followed by 35 cycles of 94°C for 60 s, 61°C for 60 s and 72°C for 180 s, the final cycle 72°C for 7 min; for *GH* - 94°C for 4 min, followed by 35 cycles of 94°C for 60 s, 64°C for 60 s and 72°C for 60 s, the final cycle 72°C for 5 min; for *PRLR* 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 55°C for 60 s and 72°C for 30 s, the final cycle 72°C for 5 min; for *MC4R* 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 62°C for 30 s and 72°C for 30 s, the final cycle 72°C for 5 min.

Restriction analysis of fragments amplified *POU1F1*, *GH*, *PRLR* and *MC4R* were performed using restriction enzymes RsaI, FokI, AluI and TaqI, respectively. Fragments were separated on a 3% agarose gel.

Table 1. Specific oligonucleotide primers for *POU1F1,* GH*, PRLR* and *MC4R*

№ Chr.	Gene	Poly morphism	Primer	PCR-frag ment	Restr	RFPL-fragments
1	*MC4R*	G1426A	5′-TACCCTGACCATCTTGATTG-3′ 5′-ATAGCAACAGATGATCTCTTTG-3′ (Kim *et al*., 2000)	226- bp	TaqI	226- 156- 70- bp
12	GH	G316A	5'-TTATCCATTAGCACATGCCTGCCAG-3' 5'-CTGGGGAGCTTACAAACTCCTT-3' (Faria *et al*., 2006)	604-bp	FokI	604- 345- 259- bp
13	*POU1F1*	C14702G	5'-AGTGTAGCCAGAGCATCT-3' 5'-ACCACATCTGCACACTCA-3' (Pierzchala *et al*., 2003)	1745- bp	RsaI	710- 388- 322-bp
16	*PRLR*	G1789A	5'-CGTGGCTCCGTTTGAAGAACC-3' 5'-CTGAAAGGAGTGCATAAAGCC-3' (Mihailov *et al*., 2014)	104 -pb	AluI	104- 85-, 59- 19- bp

Statistical Analysis

Analyses of gene effect in the observed traits were examined using a linear model:

$$Yijk = \mu + Gi + eij$$

where, Yijkl –the observed trait (The number of Days to 100-kg (Days to 100 kg), Average Daily Gain (ADG), Length of Body (LB) and Backfat Thickness (BFT)); μ – overall mean; G - the effect of *POU1F1*, GH, *PRLR* and *MC4R* polymorphisms on pig traits the *POU1F1* (i = EE, EF, FF), *GH* (i = AA, AG, GG), *PRLR* (i = AA, AB, BB) and *MC4R* (i = AA, AG, GG) genotypes; eijk – random residual effect.

The additive and dominance effects were calculated according to the formulas proposed by (Falconer and Mackay, 1996): A = (AA-BB)/2; d = AB-(AA+BB)/2, where a-additive effect, d-dominant effect, AA and BB-the value of homozygous genotypes, AB-the value of the heterozygous genotype.

Results

Restriction analysis of the *POU1F1* in Duroc pig breed defined the presence of three genotypes, which were presented as three monomorphic (774-, 153- and 108 bp) and three polymorphic (710 bp - allele E, 388- and 322 bp -allele F) fragments. In our group the genotypes EE, EF and FF are distributed with the same frequency (Table 2). The *GH* is presented by polymorphic fragments of 604 bp - allele A and 345- and 259 bp - allele G. The three genotypes AA, AG and GG were identified. The highest frequency is specific for the heterozygous AG genotype. The polymorphic fragments (104 bp - allele A and 85-, 59-, 19 bp - allele B) of the *PRLR* were observed in the group under study. The highest frequency presented allele A and genotype AA.

Table 2. Allele and genotype frequencies for *POU1F*, GH, *PRLR* and *MC4R* genes in Russian Duroc pigs

Gene	Genotype, %			Allele	
POU1F1	EE	EF	FF	E	F
	34.21	34.21	31.58	0.51	0.49
GH	AA	AG	GG	A	G
	20.00	60.00	20.00	0.50	0.50
PRLR	AA	AB	BB	A	B
	47.37	39.47	13.16	0.67	0.33
MC4R	AA	AG	GG	A	G
	52.63	39.47	7.89	0.72	0.28

The *MC4R* is represented by fragments of 226 bp of allele A and 156- and 70 bp of allele B. The greatest frequency exhibited the allele A and genotype AA.

The analysis of pigs' production traits (Table 3) showed significant effect of *POU1F1* polymorphism on the LB. The EE homozygous had a higher LD than the FF homozygous in Duroc pigs (a = 0.75). The significant effect of *POU1F1* polymorphism on the Day of 100 kg (a = 6.6), ADG (a = 73.5), LB (a = 1.2) and BF (a = 0.15) has not been defined.

The *GH* polymorphism exhibited an effect of genotypes on the growth traits of Duroc pigs. The significant additive effect on Days of 100 kg (a = 2.43) and dominant effect on LB (d = 0.71) were found. The impact of the genotypes *GH* on ADG and BF in the study population of Duroc pigs was not ascertained.

Our results showed that the genotype GG/*GH* was associated with best Days of 100 kg and the AG/*GH* with the smallest LB in pigs. Bižienė *et al*. (2011) also detected an association between genotype GG/*GH* and Days of 100 kg as well lowest feed consumption of 1 kg in various breed pigs. Result in commercial sows (Faria *et al*., 2006) detected that homozygous genotype GG/*GH* was responsible for greater carcass length means, lower drip loss and higher mean pH 24 h after slaughtering.

Table 3. Additive and dominance effects for the genotypes of *POU1F1*, *GH*, *PRLR*, *MC4R* genes in Russian Duroc pigs

Traits/Gene	Genotype means ± SE			Effect	
POU1F1	EE	EF	FF	a	d
Day of 100 kg	148.9±2.47	151.0±2.55	149.4±2.48	-0.25	0.9
ADG	828.5±31.31	854.7±21.81	873.0±33.88	-22.3	2.4
LB	117.6±0.62	116.9±0.79	116.1±0.46	0.75**	0.05
BF	1.2±0.08	1.2±0.05	1.2±0.04	-0.02	-0.01
GH	AA	AG	GG		
Day of 100 kg	151.71±2.36	148.38±1.65	146.85±1.88	2.43*	-0.62
ADG	820.57±47.32	863.05±21.41	875.71±38.08	-22.71	9.6
LB	118.00±0.87	116.48±0.58	117.43±0.97	0.28	0.71*
BF	1.25±0.08	1.21±0.05	1.20±0.06	0.03	-0.01
PRLR	AA	AB	BB		
Day of 100 kg	149.22±2.01	151.13±2.32	147.8±4.49	0.71	1.11*
ADG	879.61±25.24	815.53±26.72	858.4±24.95	10.61	-29.73*
LB	116.89±0.52	116.93±0.83	116.6±0.68	0.14	0.05
BF	1.21±0.05	1.23±0.06	1.2±0.05	0.003	0.01
MC4R	AA	AG	GG		
Day of 100 kg	148.5±1.89	152.8±1.85	143.33±1.93	2.58*	2.49*
ADG	853.05±25.61	848.4±25.57	857±27.02	-1.98	-2.58
LB	117.45±0.52	115.87±0.67	118±1.53	-0.27	-0.83**
BF	1.24±0.05	1.19±0.05	1.23±0.07	0.001	-0.02

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Polymorphism of *PRLR* the influence on growth trait was found in our study. The lowest growth rates exhibited pigs of heterozygous genotype AB/*PRLR*. The significant effect of *PRLR* polymorphism on the Day of 100 kg (d = 1.11) and ADG (d = -29.73) were found. The best performance of Day of 100 kg observed in pigs homozygous genotype BB/*PRLR*, but these were not significant. Different results for the Day of 100 kg were obtained by us a previous study (Mihailov *et al*., 2014) that with the best genotype AB/*PRLR* was determined in Landrace pigs.

Generally the *PRLR* can be considered as an efficient reproduction marker and can be used in breeding programs aimed at increasing prolificacy of sows, being confirmed by many researchers (Tomas *et al*., 2006; Iso-Touru *et al*., 2009; Zhang and Liu, 2010). Our study showed that polymorphism *PRLR* has no stable influence on the growth traits of pigs and the resulting effects relate to individual characteristics of the group being analyzed.

Influence of genotype *MC4R* on the growth traits of Duroc pigs identified in this study. The result showed the significant effect of genotype GG/*MC4R* on Days of 100 kg (a = 2.58). The heterozygous AG/*MC4R* had a lower Days of 100 kg (d = 2.49), ADG (d = -2.58) and LD (d = -0.83) than the homozygous in Duroc pigs. In general, our research defined the best indicators of growth traits of Duroc pigs associated with homozygous genotype GG/*MC4R*. Nevertheless, the low frequency of GG/*MC4R* genotype can be noted in the population under study probably due to the fact that in the recent selection of pigs special importance was given to reproductive indicators. Works of Leonova and Svyatogorova (2014) showed impact of AA/*MC4R* genotype on reproductive traits of pigs. Perhaps this influence has fixed the high frequency of genotype AA/*MC4R* in our population. As far as the main objective in the Duroc pigs selection is to improve the growth and meat traits so polymorphism *MC4R* can be used as a genetic marker. Our result has shown importance of increasing GG/*MC4R* genotype in the population of Duroc pigs.

Conclusion

Research of purebred Russian Duroc pigs (n = 360) showed the presence of polymorphisms in genes *POU1F1*, *GH*, *PRLR* and *MC4R*. Our findings demonstrate the influence of *POU1F1*, *GH* and *MC4R* on the growth traits and perceptivity of their use in breeding programs. Stable effect of the *PRLR* gene on growth characteristics was not established and we suppose that this gene is better to use in the programs aimed at improving reproductive traits of pigs.

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Author's Contributions

All authors equally contributed in this work.

Getmantseva Lyubov and Maria Leonova: Designed and performed experiments and wrote the paper.

Siroj Bakoev and Anatoly Kolosov: Developed analytical tools and analysed data.

Vyacheslav Vasilenko and Aleksander Klimenko: Designed and performed experiments.

Anastasia Radyuk: Collected and analyzed data.

Ethics

This article is original and contains unpublished materials. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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