The Single and Combined Effect of MC4R and GH Genes on Productive Traits of Pigs

1Lyubov Getmantseva, 1,2Maria Leonova, 1Aleksander Usatov, 1Siroj Bakoev, 1Aleksander Klimenko, 1Vyacheslav Vasilenko, 1Anatoly Kolosov, 1Nadezhda Shirockova and 2Maxim Makarenko

1Don State Agrarian University, Persianovskiy, Russia
2Southern Federal University, Rostov-on-Don, Russia

Abstract: The aim of our work is to identify the single and combined effect of MC4R and GH polymorphism genes on growth and reproduction traits of pigs. The study was carried out on 204 crossbred F1 females (♀ Landrace x ♂ Large White) of the Breeding Farm in Russia. The analyzed productive traits were: The number of Days to 100-kg (Days to 100 kg), Length of Body (LB), Backfat Thickness (BF) and Number of piglets Born Alive (NBA). The G1426A of MC4R and G316A of GH polymorphism were determined by PCR-RFLP method. The results showed a significant additive effect of MC4R (G1426A) on all analyzed traits and dominant effect on LB and NBA. With better Days to 100 kg associated genotype AA/MC4R, but the best indicators LB, BF and NBA installed for genotype GG/MC4R. The single effect of GH was determined to BF (dominant effect) and NBA (additive effect). The effect of genotype AG/GH on BF was observed in combination with genotypes of MC4R. In our studies we observed only six combinations of MC4R and GH genes instead of nine theoretically expected. The detected genotypes were following: AA/MC4R - AG/GH, AA/MC4R - GG/GH, AA/MC4R - AA/GH, AG/MC4R - AG/GH, AG/MC4R - GG/GH, GG/MC4R - GG/GH. The combination effect is defined for genotype AA/MC4R with genotypes of GH. The best means for all studied productivity traits were observed for the combination AA/MC4R-AG/GH. The combined effect of genotype AG/MC4R with genotypes of GH was not identified, but it was observed independently influence of MC4R on Days to 100 kg and NBA and GH on BF. The genotype GG/MC4R was detected only in combination with GG/GH genotype, which does not allow evaluating the combination effect of other GH genotypes.

Keywords: Combined Effect Genes, MC4R, GH, Pig, Growth, Reproduction

Introduction

The main direction of pig breeding is to provide new genetic knowledge about pig traits and focus on the identification and prediction of individual genes responsible for the reproduction, growth and meat traits (Fan et al., 2012; Yan et al., 2013; Leonova et al., 2015; Mihailov et al., 2014). Growth Hormone (GH), also known as somatotropin, is synthesized in the anterior pituitary (adenohypophysis in somatotropic cells). However, its production was found not only in the hypothalamus, but also in other parts of the nervous system and organs in the digestive tract. The GH for a long time been investigated as an anabolic hormone that regulates the metabolism, stimulates growth and overall body weight, promotes tissue regeneration and cell survival (Louveau and Gondret, 2004). Synthesis and secretion of GH is controlled somatoliberin hypothalamic hormones and somatostatin. Somatoliberin stimulates and somatostatin inhibits the secretion of GH, blocking the stimulating effect somatoliberin. The porcine GH gene was located on Chr12. A number of researches have showed the existence of an association between the polymorphism of GH gene and productive traits of pigs. There are data about GH gene polymorphism influence on conversion of the
Animals and Methods

Animals

The study was carried out on a cross F1 female (n = 204) to ♀Landrace x ♂Large White on the Breeding Farm in Russia. The farm specializes in breeding purebred pigs Landrace, Large White, Duroc and crossbred F1 (Landrace x Large White), F2 (F1xDuroc).

Studied Traits

The following productivity traits were measured: The number of Days to 100-kg (Days to 100 kg), Length of Body (LB), Backfat Thickness (BF) and Number of piglets Born Alive (NBA). The traits of LB and BF were obtained according to the results of growing up to 100 kg. The NBA was obtained of the three farrowing. All pigs were kept under identical and standard conditions.

Genotyping

Extraction 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 whole blood samples using a Diatom DNA Prep100 (Isogene Lab. Ltd, Russia). The MC4R (G1426A) and GH (G316A) polymorphism were determined by PCR-RFLP method. For the PCR we used specific oligonucleotide primers (Kim et al., 2000; Faria et al., 2006). Restriction analysis of fragments amplified MC4R and GH were performed using restriction enzymes TaqI and FokI, respectively. Fragments were separated on a 3% agarose gel.

Materials and Methods

Analysis of gene effect in the observed traits was examined using the following model:

\[ Y_{ijkl} = \mu + MC4R_i + GH_j + (MC4RxGH)_{ij} + Sk + e_{ijk} \]

where: \( Y_{ijkl} \) – the observed trait (The number of Days to 100-kg (Days to 100 kg), Length of Body (LB), Backfat Thickness (BF) and Number of piglets Born Alive (NBA)); \( \mu \) – overall mean; \( MC4R_i \) - the effect of genotype \( MC4R \) (i = AA, AG, GG); \( GH_j \) - the effect of genotype \( GH \) (i = AA, AG, GG); G (MC4RxGH)ij – interaction between genotypes \( MC4R \) and \( GH \) ((ij) = AA/MC4R-AA/GH, AA/MC4R-AG/GH, AA/MC4R-AG/GH, AG/MC4R-AA/GH, AG/MC4R-AG/GH, AG/GC4R-GG/GH, GG/MC4R-AA/GH, GG/MC4R-AG/GH, GG/MC4R-AG/GH, GG/MC4R-AG/GH); Sk - effect of sire; eijk – random error.

The additive and dominant effects were calculated for single genes \( MC4R \) and \( GH \) according to the formulas proposed by Falconer and Mackay (1996). The effect of combined genotypes of \( MC4R \) and \( GH \) genes were calculated for following groups: (1) AA/MC4R with genotype of \( GH \) (AA/MC4R-AA/GH, AA/MC4R-AG/GH, AA/MC4R-AG/GH); (2) AG/MC4R with genotype of \( GH \) (AG/MC4R-AA/GH, AG/MC4R-AG/GH, AG/MC4R-AG/GH); (3) GG/MC4R with genotype of \( GH \) (GG/MC4R-AA/GH, GG/MC4R-AG/GH, GG/MC4R-AG/GH).

Results

The frequencies of single and combined genotypes of \( MC4R \) and \( GH \) genes are presented in Table 1. In the studied population of pigs were installed three genotypes of \( MC4R \) and three genotypes of \( GH \), but the genotype AA/GH had a very low frequency.

In the first group identified two combination AA/MC4R-AG/GH and AA/MC4R-GG/GH in the second group there were three possible combinations AG/MC4R-AA/GH, AG/MC4R-AG/GH, AG/MC4R-GG/GH; in the third group had only one combination GG/MC4R-GG/GH.

The single and combined effect genotypes of \( MC4R \) and \( GH \) genes on productive traits of crossbred F1 pigs are presented in Table 2.
Backfat Thickness
determined for genotype GG/MC4R - GG/GH 161.41±1.24 124.10±0.42 12.65±0.03 13.23±0.20
AG/MC4R - GG/GH 159.24±1.07 122.23±0.51 13.41±0.04 13.06±0.18
AG/MC4R - AG/GH 159.72±1.27 121.33±0.52 12.46±0.05*² 13.16±0.28
AG/MC4R - AA/GH 159.42±1.15 122.01±0.42 13.43±0.08 12.65±0.16
AA/MC4R - GG/GH 153.24±0.86*ª 122.79±0.37*ª 13.42±0.05*ª 12.14±0.21*ª
AA/MC4R - AG/GH 156.59±1.12*¹ 123.01±0.37*¹ 13.42±0.05*¹ 12.14±0.21*ª
AA/MC4R - AA/GH 154.92±0.86*² 122.79±0.37*ª 13.42±0.05*ª 12.14±0.21*ª

Days of 100 kg

Significant impact on the Days of 100 kg is defined only for genotype MC4R. Individuals of genotype AA/MC4R had better Days of 100 kg on 6.5 days (4.1%) relative to the genotype GG/MC4R. Single effect GH for Days of 100 kg is not established. However, when combined genotype AA/MC4R with genotypes of GH significant differences were established. Pigs with combination AA/MC4R-GG/GH had better Days of 100 kg on 3.4 days (2.2%) compared to the AA/MC4R-AG/GH. Nevertheless, the combination of genotype AG/MC4R with genotypes of GH showed no significant differences. In the third group, all individuals have a combination of genotype GG/MC4R - GG/GH and the lowest means of Days of 100 kg.

Length of Body

The single effect MC4R gene on LB was determined. Pigs of genotype GG/MC4R were longer on 1.6 cm (1.3%) compared genotype AA/MC4R. It was also determined that pigs heterozygous genotype AG/MC4R had smaller LB on 1.3 cm (1.1%) compared to homozygous. The significant single effect of GH gene on LB undefined. The combination of genotype AG/MC4R with genotypes of GH (AA, AG and GG) was observed at the smallest length for AG/MC4R-AG/GH, but the differences were not significant. The largest LB determined for genotype GG/MC4R.

Backfat Thickness

The significant single effects on BF were attributed for MC4R and GH genes. The minimum mean of BF was established for individuals of genotype GG/MC4R and AG/GH. Pigs of genotype GG/MC4R were lower BF on 0.77 mm (6.1%) compared to AA/MC4R. The BF for pigs of heterozygous genotype AG/GH was lower on 0.81 mm (6.4%) relative to homozygous of GH. At the same time the effect of genotype AG/GH clearly observed when combined with genotype AA/MC4R and AG/MC4R. The effect of genotype AG/GH, associated with less BF, in the first group for a combination of AA/MC4R-AG/GH and the second group of AG/MC4R-AG/GH were 1.15 mm (8.2%) and 0.96 mm (7.7%), respectively.

Number of Piglets Born Alive

Polymorphisms of MC4R and GH genes had significant single gene effects on NBA. Pigs of genotype GG/MC4R were more NBA on 1.0 piglet (7.3%) compared to the AA/MC4R and heterozygous AG/MC4R was significantly higher than homozygotes on 0.5 (3.5%). Also significant effect on the NBA has been set for the genotypes of GH. With the best mean of NBA associated genotype AA/GH (+0.5). The lowest NBA was genotype AA/MC4R. However, when combined genotype AA/MC4R with genotypes AG/GH and GG/GH identified significant differences. The combined AA/MC4R-AG/GH was NBA higher on 1.2 (10.1%) compared to the AA/MC4R-GG/GH. No significant differences in NBA combined genotype AG/MC4R with genotypes of GH were observed.

Discussion

The conducted research allowed identifying the single and combined effect of MC4R and GH genes on Days to 100 kg, LB, BF and NBA.
The results showed a significant additive effect of genotypes $MC4R$ gene on all analyzed traits and the dominant effect on LB and NBA. Genotype AA/$MC4R$ was associated with better Days to 100 kg, but more efficient values of LB, BF and NBA were observed in GG/$MC4R$ genotype. In our study also established the positive effect of genotype GG/$MC4R$ on the NBA mean. The significant single effect of GH on BF (dominant) and NBA (additive) was detected. It is interesting to note that the effect of AG/GH genotype on BF also was determined in combination with genotypes of $MC4R$.

Previously research on pure-bred Landrace pigs showed a positive effect of AG/$MC4R$ on Days to 100 kg and crossbred pigs of GG/$MC4R$ had increased BF values (Klimenko et al., 2014). Most previous data have shown that A allelic variant of $MC4R$ is associated with best value of Days to 100 kg, however G allele and especially GG/$MC4R$ genotype is associated with improved BF and meat traits (Dvořáková et al., 2011; Munoz et al., 2011; Van den Broeke et al., 2015).

However, there are own peculiarities for each population. This may be due to different combinations of genes and their effects. The analysis of multiple genes ($LEP$, $LEPR$, $PIK3C3$ and $VRTN$) effects on production traits in Duroc pigs showed independently influence on growth rate and fat deposition (Hirose et al., 2014). We studied the combined effect of the $MC4R$ and GH genes in our population of nine possible genotypes were present only six: AA/$MC4R$-AG/GH, AA/$MC4R$- GG/GH, AG/$MC4R$-AA/GH, AG/$MC4R$-AG/GH, AG/$MC4R$-GG/GH, GG/$MC4R$-GG/GH.

The combination effect was observed between AA/$MC4R$ and genotypes of GH. The best indicators for all four studied productivity traits were achieved with the combination AA/$MC4R$-AG/GH. The combined effect for genotype AG/$MC4R$ with genotypes of GH was not determined and only independent influences of $MC4R$ genotypes on Days to 100 kg, NBA and GH on BF were identified. To evaluate the stability of the combined effect between AA/$MC4R$ and different allelic variants of GH, as well as finding other informative combinations further investigations need to be conducted on other breeds and crosses.

**Conclusion**

Our study has demonstrated the potential effect of the $MC4R$ and GH genes and their different allelic combinations on growth and reproduction traits of crossbred pigs. We suggest that $MC4R$ (G1426A) and GH (G316A) polymorphisms may be used in selection programs to improve economical traits in pigs.

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**Author’s Contributions**

All authors equally contributed in this work.

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

**Siroj Bakoev, Aleksander Usatov and Anatoly Kolosov:** Developed analytical tools and analysed data.

**Vyacheslav Vasilenko and Aleksander Klimenko:** Designed and performed experiments.

**Nadezhda Shirockova and Maxim Makarenko:** 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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