Leukemia Inhibitory Factor (LIF) Gene Polymorphism and its Impact on Reproductive Traits of Pigs

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Article history
Received: 4-09-2015
Revised: 17-09-2015
Accepted: 16-10-2015

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Abstract: The purpose of this study was to determine the frequencies of alleles and genotypes of the Leukemia Inhibitory Factor (LIF) gene (ID: 399503) in Landrace, Large White and Duroc pigs in Russia and to evaluate the impact on productive traits of pigs. Polymorphism was genotyped using PCR-RFLP method. In the course of studies we observed a different distribution of allele and genotype frequencies of different breeds. In all breeds under study all three genotypes AA, AB, BB were determined. The results of this study suggest that the LIF gene promotes the reproductive traits of pigs. The AA genotype sows compared to BB genotype analogues have better Total Number of Born (TNB) and Number Born Alive (NBA) by 1.4 and 1.3 (p<0.01) in Landrace sows, 1.3 and 0.9 (p<0.05) in Large White and 2.0 and 3.3 (p<0.001) in Duroc, respectively. The AB genotype sows exhibit intermediate values, which testifies the concentration of a favorable A allele in the pig genotype of animals contributing to the fertility. Our research revealed a positive effect of A allele and AA genotype that indicates the possibility to use this polymorphism in improving reproduction traits of sows.

Keywords: LIF, Gene, Polymorphism, PCR-RFLP, Pig, Reproductive Traits

Introduction

Leukemia inhibitory factor is a cytokine of pleiotropic action, involved in many physiological processes, including proliferation, differentiation and cell survivability, i.e., it exerts its impact on fecundity (Drogemüller et al., 2001; Rodriguez-Zas et al., 2006; White et al., 2007; Mihailov et al., 2014). Direct evidence of LIF expression in endometrium, being essential for implantation process, was obtained in experiments on mice using genetic engineering. The works of C. Stewart et al. (1992) proved that in female mice with an inactivated LIF-gene the process of ovum fertilization was not disrupted, but those were not able to embryo implantation. The artificial introduction of recombinant LIF to mice with LIF gene deficit regenerated the implantation process (Mikołajczyk and Metkalf, 1991). Recent studies of Polish scientists have proved the relations between mutations in the LIF gene and women infertility (Králíčková et al., 2006). Due to its functions, LIF gene is seen as a fertility candidate gene for many mammalian species, including pigs (Spötter et al., 2001; Lopez et al., 2006; Fan et al., 2009; Ropka-Molik et al., 2012).

Pig LIF is localized in the chromosome 14 (NC_010456.4 (50263470..50277210)) within the QTL confidence interval, associated with the Total Number of Born (TNB) and Number Born Alive (NBA).

The aim of our study was to determine the frequencies of alleles and genotypes LIF in Landrace, Large White and Duroc pigs in Russia and estimate the impact of SNP (rs3463076786:C>T) on productive traits of pigs.

Materials and Methods

Animals

For a total, the analysis included 510 purebred pigs developed to Breeding Farm «Yubleiny» in Russia. Among them there are Landrace (n=329), Large White (n=135) and Duroc (n=46). The farm specializes in breeding purebred pigs Landrace, Large White and Duroc. The Landrace and Large White breeding aimed at improving the reproductive traits and Duroc- on growth and meat. All pigs were kept under identical and standard conditions.
The Study of Productive Traits

Reproductive traits were taken into account in sows Landrace (n=210), Large White (n=68) and Duroc (n=34). The type of covering sows was artificially insemination. Reproductive traits studied were: Total Number of Born (TNB), Number Born Alive (NBA) and Litter Weight at Birth (LWB). The data of the first three farrowing were taken for the analyses. The average values of reproductive traits of sows are shown in Table 1.

Genotyping

Polymorphism was genotyped using PCR-RFLP method. DNA was isolated from blood leukocytes using a Diatom DNA Prep100 (Isogene Lab.Ltd.Russia). For the PCR we used specific oligonucleotide primers (Spötter et al., 2001):

5’-ATGTGGATGTGGCCTACGG-3′ (GenBankAJ296176, nucleotide 6842-6861);
3’-GGGAACAAGGTGGTGATGG-5′ (GenBank AJ296176, nucleotide 7231-7249)

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 (Evrogen, Russia). Conditions were 94°C for 4 min, followed by 30 cycles of 94°C for 30 s, 58°C for 60 s and 72°C for 30 s. The PCR-RFLP analysis of the LIF fragment length of 407 bp was performed using the DraIII endonuclease and separated on a 2% agarose gel with the addition of ethidium bromide. A 407-bp fragment was observed for the A allele and the AA genotype, 266- and 144-bp fragments for the B allele and the BB genotype and 407-, 266- and 144-bp fragments for A and B alleles and the AB genotype (Fig. 1).

Statistical Analysis

Analysis of gene effect in the observed symptoms was examined using a linear model:

\[ Yijkl = \mu + Gi + Sexj + LIFk + eijkl \]

Where:
- \( Yijkl \) = The phenotypic record
- \( \mu \) = The general mean
- \( Gi \) = The effect of genetic group of sow
- \( Sexj \) = The effect of sex (j = F, M)
- \( LIFk \) = The effect of LIF genotype (k = AA, AB, BB)
- \( eijkl \) = The random error

Results

We tested 329 Landrace pigs, 135 Large White pigs and 46 Duroc pigs on the presence of LIF polymorphism by means of PCR-RFLP method. Allele and genotype frequencies of LIF for Landrace, Large White and Duroc pigs are shown in Table 2. All three genotypes by the LIF (AA, AB and BB) were determined in all groups under study. In Landrace groups the highest frequency was exhibited by B allele (0.56).
The analysis of the genetic structure of the Large White pigs indicated that low frequency was typical for the BB genotype (5.9%) and the AA genotype’s frequency had an intermediate value (36.3%). However, in evaluating the frequency distribution in the given population the clear priority was typical for the A allele with frequency of 0.65, while B allele frequency being 0.34. At Duroc pigs the highest frequency was typical for the B allele (0.76), the frequency of A allele was 0.23. AA genotype was less frequent (8.7%) and BB genotype was 60.9%.

The results of studying impact of LIF genotypes on reproductive traits are presented in Table 3.

Our results showed a significant effect of LIF polymorphism on reproductive traits of pigs. In all breed groups sows with genotype AA/LIF had the best TNB and NBA. Effect genotype AA/LIF compared BB/LIF on TNB and NBA were +1.4 and 1.3 (p<0.01) in Landrace sows, +1.3 and 0.9 (p<0.05) in Large White and +2.0 and 3.3 (p<0.001) in Duroc, respectively. The AB genotype sows exhibit intermediate values, which testifies the concentration of a favorable A allele in the pig genotype of animals contributing to the fertility.

Discussion

As a result of studies of allele frequencies in the German synthetic line of pigs by LIF (Spött et al., 2001) a very low frequency of A allele (0.27) was established. The research of Polish scientists (Napierala et al., 2014) demonstrated the higher presence of B allele (0.64) as compared to A allele (0.36), resulting in a relatively high effect on the BB genotype frequency (0.44) compared to the second homozygous AA genotype (0.15), whereas the heterozygous AB genotype, had frequency of 0.41. In addition, the homozygous genotypes were more frequent (0.59) than the heterozygous ones (0.41).

These frequencies of alleles and genotypes are consistent with the results of Spött et al. (2005), who carried out an investigation of the German synthetic line of pigs. However, other studies of Spött et al. (2009) carried out on a larger number of different breeds of sows showed a different distribution of the both alleles and genotypes compared to their first study.

Compared to the distribution of allele frequencies in our study, the frequency of alleles and genotypes in German Landrace sows showed significant differences. The A allele occurs with frequency of 0.56, B allele - 0.44. Among the genotypes, the AB genotype had the highest frequency of 0.54, the AA genotype -0.34 and BB genotype -0.12, which is consistent with our outcomes for the Landrace and Large White breed. Allele frequencies in German Large White sows differed from the previous population. The A allele was less frequent (0.25) than allele B (0.75). BB genotype was most prevalent (0.51) and AA genotype occurred less (0.06), which is consistent with our results for the Duroc breed.

Trinidad (2014) in his study of different pig breeds by LIF in the Philippines showed the distribution of frequencies and genotypes as follows; frequencies of AA, BB and AB genotypes were 0.26, 0.30 and 0.44, respectively. In the Philippine pig population the frequency of A allele and B allele were almost equal and amounted to 0.48 and 0.52, respectively. The outcomes of study of Landrace × Yorkshire pigs show that the highest frequency was exhibited by AB genotype (0.73), the frequency of BB genotype was 0.12. Recent studies on the...
Polish Large White and Landrace breeds showed that AA genotype occurred rarely (0.13) and the frequency of AB genotype (0.45) was only slightly higher than that of BB genotype (0.42) (Mucha et al., 2013).

The impact of LIF genotypes being studied Spötter et al. (2006) obtained the results in which the sows of the synthetic German line, despite the low frequency of the A allele, exhibited a positive effect of the recessive genotype (0.42) (Mucha et al., 2006). The subsequent research of Spötter et al. (2009) included two large populations of Large White and Landrace lines and showed that sows with AB genotype of both breeds have the highest number of born alive in the first litter, which is consistent with our results.

Our results as well as those of other researchers prove that the consolidation of the favorable genotype contributes to the reproductive traits of different breed pigs. Therefore polymorphism is possible to offer as a genetic marker of reproductive traits of pigs. Animal testing for the presence of the desired of AA genotype will allow in the early stages selected pigs genetically susceptible to high reproductive performance.

Conclusion

The results of this study suggest that the LIF gene promotes the reproductive traits of pigs. Our research revealed a positive effect of A allele and AA genotype that indicates the possibility to use this polymorphism in improving reproduction traits of sows. Thus, these studies confirm the role for LIF polymorphism in improving economically important characteristics of Landrace pigs in Russia. These results contribute to the current state of knowledge about the genetic factors that determine the level of productive qualities of pigs and to use them in practical breeding pigs.

Acknowledgement

This research was supported by the Russian Ministry of Education and Science, project no. 40.91.2014/K.

Author’s Contributions

All authors equally contributed in this work
Leonova M.A. and Getmantseva L.V.: Designed and performed experiments and wrote the paper
Vasilenko V.N. and Klimenko A.I.: Designed and performed experiments.
Usatov A.V., Bakoev S. Yu, Kolosov A. Yu.: Developed analytical tools and analysed data.
Shirockova N.V.: Collected and analysed 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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