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Short Report

Effect of the polymorphism in *GPX5* gene on reproductive performance traits in Large White x Landrace crossbreed sows

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The aim of the experiment was to detect polymorphism in *GPX5* gene encoding glutathione peroxidase 5 and to determine associations between individual genotypes and following reproductive traits in the Polish Large White x Landrace crossbred sows (n=442): total number of piglets born (TNB), number of piglets born alive (NBA) and number of piglets weaned (NW). The polymorphism in *GPX5* gene was detected using the PCR-RFLP method with specific primers and restriction enzyme *Hinf*I. Two different alleles of the *GPX5* gene were identified – *1B* (0.42) and *2B* (0.58). Genotype distribution was in a state of Hardy-Weinberg equilibrium. The association analysis showed significant (P≤0.01) differences between sows carrying different genotypes and TNB, NBA, NW. The *1B1B* genotype was favourable for all analyzed reproductive traits in first parity. In later parities similar tendency was observed, but differences were statistically not significant.

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For pork production it is very important to obtain numerous offspring from each sow in successive litters. Reproduction abilities are quantitative traits and depend on genetic and environmental factors. Currently, two approaches are applied in the identification of QTL in animals: mapping genes by linkage analysis and evaluation of the effect of polymorphisms in candidate genes for the trait of interest.

Studies carried out by many authors [Mote *et al*. 2009, Marantidis *et. al*, 2013, Fang *et al.* 2014] suggest that certain genes can be used as markers for reproductive traits in pigs. The most relevant candidate genes for reproductive traits include: estrogen receptor gene (*ESR*); follicle-stimulating hormone beta subunit gene (*FSHB*); leptin and leptin receptor genes (*LEP* and *LEPR*); prolactin and prolactin receptor genes (*PRL* and *PRLR*); osteopontin (*OPN*); epidermal growth factor (*EGF*); amphiregulin (*AREG*); retinol binding protein 4 (*RBP4*); glutathione peroxidase 5 (*GPX5*) and several other genes (for review – see Onteru *et al*. 2009).

One of them – *GPX5* gene encodes an epididymis-specific enzyme in mice, rats, pigs, monkey as well as in human. GPX5 belongs to the noncanonical selenium-independent GPX. It has the highest aminoacids homology to GPX3. Together with GPX3, GPX5 represent more than 95% of epididymal GPX mRNA and protein [Brigelius-Flohé and Maiorino 2013]. GPX5 acts as a true reactive oxygen species (ROS) scavenger protecting epididymis-transiting sperm cells from ROS-mediated loss of integrity [Noblanc *et al*. 2011]. It was confirmed inter alia by Noblanc *et al*. [2012] who reported that spermatozoa in mice lacking activity of the sperm nucleus glutathione peroxidase 4 (snGPX4) and GPX5 display sperm abnormalities for example delayed and defective nuclear compaction, nuclear instability and DNA damage.

In pig, *GPX5* gene is localized in the region in which several quantitative trait loci (QTL) for reproductive traits were detected such as uterine capacity, ovulation rate and litter size. It is also known that this gene is situated within the swine major histocompability complex (SLA) which is assigned to chromosome 7 [Bertani *et al*. 1999] and has been suggested to exert an effect on reproductive traits in swine [Vaiman *et al*. 1998, Buske *et al*. 2005]. The structure of the porcine *GPX5* gene is only partially described and an analysis of homology between its fragment (GenBank, acc. no.AF124818), described by Bertani *et al*. [1999] and the human sequence (GenBank, acc. no. NM 003996) revealed that the two polymorphic sites are present in intron 1 in this gene. There are substitution A>G (alleles *1B* and *2B* recognized by *Hinf*I restriction enzyme) and deletion/insertion of 522 bp (alleles *1C* and *2C*) [Bertani *et al*. 1999].

The aim of this study was to identify A>G polymorphism in *GPX5* gene in Large White x Landrace sows as well as to study possible associations between individual genotypes and some reproductive traits.

Material and methods

The experimental population included 442 Polish Large White x Landrace crossbred sows. They were bred and raised at a farm in Western Pomerania (Poland). Rearing and feeding conditions were similar for all animals.

Genomic DNA was isolated from blood leukocytes using Master Pure Kit (Epicentre Technologies, USA). Genotypes were determined using the PCR-RFLP procedure according to Bertani *et al*. [1999]. PCR mix included: 1xPCR buffer, 2.5mM $MgCl_2$, 0.2mM dNTPs, 1.2mM of each primer, 70-90ng of genomic DNA, 1U of *Taq* DNA polymerase (MBI Fermentas, Germany) and sterile deionised water filled up to volume 20µl. Reactions were performed using the following thermal profile: 94°C for 3min followed by 40 cycles of 40s at 94°C, 45s at 52°C, 2.5min at 72°C and a last extension for 5 min at 72°C. Digestion of the PCR product was performed with 5U of *Hinf*I restriction endonuclease (MBI Fermentas) at 37°C overnight. The restriction fragments were separated by electrophoresis in 2% agarose gel stained with ethidium bromide at 0.5 µg/ml, subsequently visualized and recorded with the use of the Vilber Lourmat system (France).

The following performance traits were analyzed: number of piglets born (TNB), number of piglets born alive (NBA), number of piglets weaned (NW). All data were analyzed with the REML procedure of SAS/STAT (9.1.0) [2004] on basis of the unitrait mixed linear model:

$$y_{ijklm} = \mu + G_i + YS_j + P_k + d_l + s_m + e_{ijklm}$$

where:

y_{ijklm} – *ijklm*-th observation;

μ – overall mean;

G_i – fixed effect of *i*-th genotype at *GPX5* ($i = 1, 2, 3$);

YS_j – fixed effect of *j*-th year and season ($j = 1, 2, \ldots, 20$) – (5 years$_*$4 seasons);

P_k – fixed effect of *k*-th the parity ($k = 1, 2, \geq 3$);

d_l – random polygenic effect of the *l*-th dam ($l = 1, 2, \ldots, 297$);

s_m – random polygenic effect of the *m*-th sire ($k = 1, 2, \ldots, 44$);

e_{ijklm} – random error connected with *ijklm*-th observation.

Following population parameters were calculated with the use of PowerMarker ver. 3.25 [Liu and Muse 2005]: genotypes and alleles frequency, mean heterozygosity expected heterozygosity (gene diversity), polymorphic information content (PIC) and Hardy-Weinberg equilibrium (χ^2).

The *RYR1* genotypes were established using primer sequences designed by Kamiński *et al*. [2002].

Results and discussion

Two different alleles of the *GPX5* gene were identified: alleles *1B* and 2*B* that control the occurrence of three genotypes: *1B1B* and *1B2B* and *2B2B*. Allele *1B* occurred with a frequency of 0.42 and allele *2B* with a frequency of 0.58. The *1B1B* genotype occurred with a frequency of 0.19, *1B2B* of 0.46, and *2B2B* of 0.35. Values for mean heterozygosity and expected heterozygosity were as follows: 0.46, 0.49, however the PIC value amounted 0.37. Analyzed population was in the state of genetic equilibrium (χ^2 = 1.7766, p-value = 0.1826).

Our previous studies as well as those carried out by other authors showed that the *RYR1* genotype significantly affected many economically important traits: reproductive traits [Terman and Kumalska 2012], carcass and meat quality traits [Urbański *et al*. 2013], and could modify the effect of other genes. Therefore in the present study we have also analysed the *C*1843*T* transition of the *RYR1* gene in all animals. Only one genotype was observed at the *RYR1*/*HinP1*I *locus*. The material analyzed appeared to be free of *RYR1*^{T} allele and the effect of *RYR1* genotype was not included into the statistical model.

The effects of the *GPX5* gene genotypes on the reproductive traits of the Large White x Landrace sows are given in Table 1. The sows with the *1B1B* genotype had largest litter sizes, *2B2B* smallest, and *1B2B* moderate and the difference between them was statistically significant (P≤0.01) only in the first parity.

Table 1. Effects of the *GPX5* genotypes on reproductive traits of Large White x Landrace crossbred sows – least square means and their standard errors

Genotype	Parity	n	TNB		NB		NW	
			LSM	SE	LSM	SE	LSM	SM
1B1B	I	82	9.69^a	0.28	9.53^a	0.29	9.47^a	0.28
1B2B		197	8.91^b	0.19	8.78^b	0.19	8.63^b	0.20
2B2B		153	8.78^b	0.21	8.62^b	0.21	8.54^b	0.20
1B1B	II	80	9.98	0.26	9.87	0.27	9.79	0.28
1B2B		200	9.75	0.20	9.67	0.19	9.58	0.22
2B2B		154	9.61	0.24	9.57	0.23	9.51	0.23
1B1B	≥III	328	10.17	0.23	10.03	0.23	9.93	0.23
1B2B		662	9.94	0.18	9.87	0.17	9.65	0.18
2B2B		458	9.71	0.20	9.64	0.21	8.51	0.21

^{ab}Means within column bearing different superscripts differ significantly at P≤0.01, n – number of sows within parity,
TNB – number of piglets born; NBA – number of piglets born alive; NW – number of piglets weaned.

From the physiological point of view, *GPX5* is involved mainly in sperm quality and it seems that there is no direct connection between its function and litter size in sows. Vaiman *et al*. [1998] and Buske *et al*. [2005] speculated about the physiological influence of chromosomal region flanking *GPX5* gene – the MHC class III on reproductive parameters. In the population investigated by Buske *et al*. [2006], however, no association was observed between litter size and individual genotypes of

the *GPX5* gene. In their opinion *GPX5* is rather involved in paternal, than in maternal fertility. Dall'Olio *et al*. [2012] analyzed association between five DNA markers and the number of functional teats (FTNUM) in the Italian Large White sows. One of the investigated markers was a SNP localized in the 3'UTR of *GPX5* gene. Based on FTNUM the sows were divided into 2 categories: having 14 or more than 14 teats. Analysis showed statistically significant ($P<0.01$) differences in the allele frequency of the *GPX5* gene between these two groups. The authors also emphasized lack of relation between the GPX5 function and performance traits in sows. They proposed that the association between *GPX5* gene and reproductive traits may be due to linkage disequilibrium with a functional allele within another gene, on chromosome 7. Taking into consideration the assumption of Vaiman *et al*. [1998] and Buske *et al*. [2005] it could be one of the genes from the MHC class III.

Summarizing, the results of the current study demonstrated that the polymorphism in intron 1 of the porcine *GPX5* gene, identified by the *Hinf*I enzyme, could be important in terms of certain reproductive traits in the studied crossbred pigs. Statistically significant ($P\leq0.01$) relationships between individual genotypes and the traits values (especially the number of piglets born and weaned in the first parity) were observed. Therefore the *GPX5* gene may be considered as a candidate gene for reproductive traits in sows.

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