Restriction polymorphism $FABGL/BbvI$ in herd of sows derived from crossing Polish Large White x Polish Landrace breeds

Abstract
The $FABGL$ gene encodes a nicotinamide adenine dinucleotide (NAD)-dependent 17$\beta$-hydroxysteroid dehydrogenase (17b-HSD) which is responsible mainly for immune response. It can also regulate the concentration of biologically active estrogens and androgens. It is appear as oxidative enzyme that inactivates estradiol, testosterone, and dihydrotestosterone as well as reductive enzyme by synthesis of estradiol from estrone. Investigations showed that $FABGL$ gene is expressed within the ovaries and testes. Different variants of this gene may be associated with reproduction traits in pigs. The aim of this study was to determine polymorphism in the promoter region of $FABGL$ gene as well as examine associations between particular genotypes and reproduction traits in Polish Large White x Polish Landrace sows. Polymorphism in $FABGL/BbvI$ was determined by applying PCR-RFLP and following frequency of alleles were obtained: $A$ - 0.45, $B$ - 0.55. Statistical analysis showed associations ($P\leq0.05, P\leq0.01$) between particular genotypes and some reproduction traits in investigated herd of sows.

Key Words: $FABGL$ gene, sows, polymorphism, reproduction traits

Zusammenfassung
Titel der Arbeit: Restriktionspolymorphismus des $FABGL/BbvI$ Gens bei Kreuzungssauen aus Große Polnische Weiße x Polnische Landrasse

Schlüsselwörter: $FABGL$-Gen, Sau, Polymorphismus, Wurfleistungsmerkmale

Introduction
Steroid hormones act through specific receptors effect activation of gene transcription. The biological activity of these hormones is regulated at the pre-receptor level (MINDNICH et al., 2004). Several enzymes are committed in this process. Nicotinamide adenine dinucleotide (NAD)-dependent 17$\beta$-hydroxysteroid dehydrogenases (17$\beta$-HSD) are key enzymes acting in the last step of formation of androgens and estrogens. Eleven 17$\beta$-HSDs coded by different not homologous genes have been discovered, which vary in tissue distribution, catalytic preferences, substrate specificity, subcellular localization and mechanism of regulation. These enzymes are mainly involved in conversion at position 17 of sex steroids. They can also metabolize
different substrates including alcohols, bile acids, fatty acids and retinols (ADAMSKI and JAKOB, 2001). 17β-hydroxysteroid dehydrogenase VIII (17β-HSD8) protein efficiently catalyses the oxidation of estradiol, testosterone and dihydrotestosterone leading to their inactivation. It also appear reductive activity by synthesis of estradiol from estrone (FOMITCHEVA et al., 1996). Gene which codes this enzyme is known as FABGL gene as well as KE6 or RING2 gene. In swine it was assigned to chromosome 7, within the class II region of pig major histocompatibility complex. It is called SLA and located on the long arm in 7q1.1 (CHARDON et al., 1999). FABGL gene consist of nine exons with 86% and 82% homology to human and mouse mRNA sequences respectively (JACOBS et al., 2002). FOMITCHEVA et al. (1996) have demonstrated that FABGL gene is expressed within the ovaries and testes maintaining local level of sex steroids. Polymorphisms in this gene may be associated with the fertility and lipid metabolism in swine.

The aim of this study was to determine polymorphism in the promoter region of FABGL gene discovered by JACOBS et al., (2002) as well as examine associations between particular genotypes and reproduction traits in Polish Large White x Polish Landrace sows.

**Materials and methods**

Investigation were carried out on herd 305 sows derived from crossing Polish Large White x Polish Landrace breeds. Conditions of rearing and feeding were equalized for all animals. DNA was extracted from whole blood by using standard kit (MasterPure™ DNA Purification Kit for Blood, Epicentre). Primers for PCR reaction were designed according to JACOBS et al., (2002). To estimate optimal annealing temperature gradient thermocycler was used (TGradient, Biometra). PCR was performed for each sample in total volume 15µl contains: 1xPCR buffer (C), 2mM MgCl2, 0.2mM dNTP mix, 15pmol of each primer, 0.75U Taq (Eurx), about 80ng of DNA, PCR grade water up to 15µl. Following thermal profile was applied: initial denaturation at 94°C for 5min, 35 cycles: 94°C/45s, 69°C/50s, 72°C/40s and the final extension at 72°C for 5min. 5µl of PCR product was checked on 1% agarose gel staining with ethidium bromide. After positive estimation it was digested by using 1U of BseXI (BbvI) restriction enzyme in 65°C overnight. Restriction fragments were separated on 2% agarose gels. To visualization and record gels Vilber Lourmat system was used.

The analysis of associations between FABGL/BbvI genotypes and total number born (TNB), number of weaned (NW) and number falls (NF) were performed applying following models according to parity order:

First parity (I)

\[ Y_{ijk} = \mu + a_i + c_j + d_k + b(\text{av}) + e_{ijk} \]

Second and remaining (>I)

\[ Y_{ijk} = \mu + a_i + c_j + d_k + e_l + b(\text{av}) + e_{ijk} \]

where:

- \( Y_{ijk} \) - analyzed trait
- \( \mu \) - the overall mean
- \( a_i \) - constant effect of FABGL/BbvI genotype (i = 1, 2, 3)
- \( c_j \) - fixed effect of sire (j = 1, ...38)
\(d_k\) - fixed effect of year-season (\(k = 1, \ldots, 14\))
\(e_l\) - fixed effect of parity order (\(l = 2, \ldots, 14\))
\(b_{(av)}\) - the regression of age at first farrowing/value of trait
\(e_{ijk}\) - the random error

Results
Two alleles of \(FABGL\) gene were identified in investigated herd of sows: \(A\) and \(B\). Presence of three genotypes: \(AA, AB, BB\) were confirmed. The following lengths of restriction fragments were observed: \(AA - 273\)bp, \(AB - 273, 150, 123\)bp, \(BB - 150, 273\)bp. Table 1 presents obtained frequency of alleles and genotypes of \(FABGL/BbvI\). The analysis of associations between polymorphism at \(FABGL/BbvI\) locus and reproduction traits is showed in Table 2.

Table 1
Frequency of the \(FABGL/BbvI\) alleles and genotypes

<table>
<thead>
<tr>
<th>N</th>
<th>(FABGL/BbvI) alleles</th>
<th>Frequency</th>
<th>(FABGL/BbvI) alleles</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>59</td>
<td>(AA)</td>
<td>0.19</td>
<td>(A)</td>
<td>0.45</td>
</tr>
<tr>
<td>158</td>
<td>(AB)</td>
<td>0.52</td>
<td>(B)</td>
<td>0.55</td>
</tr>
<tr>
<td>88</td>
<td>(BB)</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Means and standard deviations of investigated traits in relation to \(FABGL/BbvI\) genotypes

<table>
<thead>
<tr>
<th>(FABGL/BbvI) genotypes</th>
<th>Litter</th>
<th>n</th>
<th>TNB(^1)</th>
<th>NW(^2)</th>
<th>NF(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>I</td>
<td>55</td>
<td>8.96±2.03</td>
<td>7.76±1.92</td>
<td>1.091±0.369</td>
</tr>
<tr>
<td>AB</td>
<td>&gt;I</td>
<td>137</td>
<td>8.93±2.21</td>
<td>7.73±1.83</td>
<td>1.022±0.426</td>
</tr>
<tr>
<td>BB</td>
<td></td>
<td>75</td>
<td>8.68±2.15</td>
<td>7.72±2.06</td>
<td>0.027±0.162</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>267</td>
<td>8.87±2.15</td>
<td>7.73±1.91</td>
<td>0.713±0.319</td>
</tr>
<tr>
<td>AA</td>
<td></td>
<td>233</td>
<td>10.32±2.54</td>
<td>8.80±2.16(^{n})</td>
<td>0.537±0.770</td>
</tr>
<tr>
<td>AB</td>
<td></td>
<td>660</td>
<td>10.00±2.43</td>
<td>8.45±2.06(^{a})</td>
<td>0.441±0.705</td>
</tr>
<tr>
<td>BB</td>
<td></td>
<td>435</td>
<td>9.77±2.58(^{a})</td>
<td>8.39±2.22(^{b})</td>
<td>0.375±0.820</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1328</td>
<td>9.98±2.51</td>
<td>8.49±2.13</td>
<td>0.451±0.765</td>
</tr>
</tbody>
</table>

Means in lines with the same letters differ significantly, small letters - \(P<0.05\), capitals - \(P<0.01\)

\(^1\)TNB - total number born, \(^2\)NW - number of weaned, \(^3\)NF - number of falls

Discussion
There are many investigations concerning polymorphisms and their relationships with performance traits in domestic animals. In pigs mainly reproduction, carcass and meat quality traits are taking under consideration. Recent studies have proved that polymorphisms in some genes may be associated with reproduction traits in pigs. In sows have examined following genes: \(ESR1, FSHB\) (WANG et al., 2006, HUMPOLIČEK et al., 2006), \(RBP4\) (WANG et al., 2006), \(CYP21\) (ZIEMAK and GRZESIAK, 2006), \(GPX, FUT1, ESR2\) (BUSKE et al., 2006) \(PRLR, LEP\) (TERMAN 2006) \(sFBP\) (VALLET et al., 2005), \(EPOR\) (VALLET et al., 2005), however in boars: \(ESR1, ESR2\) (TERMAN et al., 2006), \(GNRHR, PRL, PRLR, FSHB, LHB, FST, INHA, INHBA, INHBB\) (LIN et al., 2006) \(ACTN1, ACTN4, ACTG2\) (WIMMERS et al., 2005). In our study we investigated \(FABGL\) gene on account of role in reproduction which
plays the product of this gene. We have found statistical significant associations between particular genotypes and total number born and number of weaned in second and remaining parities. Higher value for TNB characterized sows (P≤0.01) with genotype \textit{AA} (10.32) in relation to genotype \textit{BB} (9.77). Considering NW, higher value for this traits achieved sows with genotype \textit{AA} (8.80) than sows with genotype \textit{BB} (8.39) (P≤0.01) and \textit{AB} (8.45) (P≤0.05). Differences in number of falls for \textit{FABGL/Bbv1} genotypes were also observed but they were not statistical significant. There are no data in the literature concerning association study of \textit{FABGL} gene. Only JACOBS et al. (2002) has given frequency of alleles in different breeds of pigs. In our study following frequency of alleles were obtained: \textit{A} - 0.45, \textit{B} - 0.55. Similar frequency were observed in two breeds: Czech Meat Pig \textit{A} - 0.47, \textit{B} - 0.53 and Landrace \textit{A} - 0.42, \textit{B} - 0.58. Higher frequency of allele \textit{A} were found in Pietrain - 0.57 and Large White - 0.54. Allele \textit{B} was not found in pigs belong to Meishan breed. Results of our study indicates that genotype \textit{AA} is favourable for TNB and NW. It may be suggested that \textit{FABGL} gene is 'candidate gene' for reproduction traits but investigation should be verified on bigger population as well as on different breeds.

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