ASSOCIATION BETWEEN TWO POLYMORPHISMS WITHIN INTRON 4 OF INSULIN-LIKE GROWTH FACTOR RECEPTOR TYPE 1 GENE (IGF1R/HinfI AND IGF1R/Mph1103I) AND MILK TRAITS OF POLISH HOLSTEIN-FRIESIAN COWS

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Abstract. The aim of the present study was to determine the frequency of the two polymorphic variants located in intron 4 of the gene coding for insulin-like growth factor 1 receptor (IGF1R) in the examined herd of 184 Holstein-Friesian cows and to search for the association between these polymorphisms and the selected milk performance traits. The IGF1R gene polymorphism was identified with PCR-RFLP using the HinfI and Mph1103I restriction enzymes. For the IGF1R/HinfI polymorphism, the highest frequency was found for the BB genotype (0.49), a similar one was in the case of the AB genotype (0.45) and the lowest one was for the AA genotype (0.04). The frequency of alleles was as follows: allele A – 0.28 and allele B – 0.72. Statistical analysis showed that the analysed polymorphism significantly affected milk yield, milk protein yield (P≤0.01) and milk fat yield (P≤0.05), favouring the BB genotype. For the IGF1R/Mph1103I polymorphism, no individuals with the AA genotype were recorded. A high frequency of allele B (0.94) was found. No significant effect of the IGF1R/Mph1103I polymorphic site on the yield of milk, fat and protein was shown. Statistically significant differences (P≤0.05) were observed only for the percentage content of milk fat and protein with indication on the positive effect of the BB genotype.

Keywords: IGF1R, milk traits, polymorphism, QTL

INTRODUCTION

Search for genes of major phenotypic effect is one of the most intensively developing fields of genetic and breeding research. Such genes, with regard to production traits, are called quantitative traits loci – QTL [Kurył 2000]. Due to the significant contribution of...
the IGF-I/IGF-IR system to the processes of growth and development of the mammary gland and muscle tissue, the determination of the structure and polymorphic sites in the gene coding for the insulin-like growth factor I receptor (IGF1R) can enable potential selection of individuals in respect of favourable polymorphic variants for the analysed traits. Moody et al. [1996] mapped the IGF1R gene to the bovine chromosome 21. It consists of 21 exons, divided with sometimes very long introns (even 50–150 kbp). The Whole Genome Shotgun (WGS) project for Bos taurus, that is, the sequencing of the entire genome of domestic cattle, resulted in the detection and description of over 200 polymorphic sites within the IGF1R gene. Except for IGF1 gene, detailed research on an effect of the newly discovered mutations in the remaining genes coding for the elements of the insulin-like growth factor I (IGF-I) system in connection with the composition, quality and amount of produced milk as well as the rate of an increase in muscle mass has not been conducted so far.

The aim of the study was to devise a method of detection of the two polymorphic sites (rs41960620 and rs41960621) in intron 4 of the bovine IGF1R gene, to determine the frequency of alleles and genotypes for these polymorphisms and to estimate the associations between these polymorphisms and selected milk performance traits.

MATERIAL AND METHODS

The research was conducted on a herd of Holstein-Friesian dairy cows of Black-and-White strain (184 individuals) kept in the West Pomerania Province. The data on the milk performance of cows were derived from the breeding documentation being the part of the milk recording.

Peripheral blood was taken from animals from the external jugular vein into test tubes containing the EDTA anticoagulant. MasterPure™ Genomic DNA Purification Kit from Epicentre Technologies as well as isolation procedure suggested by the manufacturer were used for the DNA isolation. In order to analyse the occurrence of polymorphism in intron 4 of the IGF1R gene in dairy cows, PCR-RFLP was carried out using the specific pair of primers:

- Forward (F) primer 5’– CTGGATATGTCCGCTTAGC – 3’
- Reverse (R) primer 5’– ACAGCTCTTGTGTCCCTGGT – 3’

The original primer sequences designed using the Primer3 software allowed amplification of the IGF1R gene fragment of 231 bp, within which two polymorphic sites submitted to NCBI with accession numbers rs41960620 and rs41960621 are located (both SNPs are transitions C→T). Both mutations has not been validated so far.

PCR was performed in the reaction mixture that contained DNA template obtained from the previous isolation from the cellular elements of peripheral blood of the examined cows, thermostable enzyme – DNA Taq polymerase (MBI Fermentas), buffer with (NH4)2SO4 (MBI Fermentas) and MgCl2 (MBI Fermentas), nucleotide mixture (MBI Fermentas), pair of primer sequences (Oligo, IBBPAN, Warsaw). The whole reaction mixture was adjusted to a final volume of 20 μl with nuclease-free deionised water (Epicentre Technologies).
The following thermal profile was applied: preliminary denaturation of DNA template at 94°C for 5 min, followed by 33 cycles: denaturation of the double-stranded DNA template at 94°C for 50 s, annealing of the primers to single-stranded template at 59.5°C (T_m) for 60 s, synthesis of complementary strands (polymerase activity) at 72°C for 50 s and final elongation at 72°C for 7 min. The reaction was carried out in the Biometra thermocycler.

Two restriction enzymes were used for the analysis of the polymorphic sites. The HindIII restriction enzyme recognizes and cuts a specific motif (G/ANTC) in the analyzed DNA fragment and the cleavage occurs at only one position. The cleavage site coincides with the occurrence of the rs41960620 mutation. In the case of Mph1103I (NsiI) restriction enzyme, this endonuclease recognizes and cuts the ATGCA/T sequence within the obtained PCR product, that coincides with the occurrence of the second SNP – rs41960621.

The 231-bp PCR product was digested with 5U of the HindIII or 5U of the Mph1103I (NsiI) restriction enzyme at 37°C for 3 h. Electrophoresis was carried out in the 2% agarose gel with ethidium bromide in the TBE buffer in the presence of the pUC19/MspI DNA mass marker (MBI Fermentas) at the constant voltage of 120 V. Restriction fragments were visualized under UV light using the Vilber Lourmat transilluminator and archived.

The statistical analysis of the association between the IGF1R/HindIII and IGF1R/Mph1103I polymorphisms and the yield of milk (kg), milk fat and protein (kg) as well as milk fat and protein content (%) was performed using the Statistica® 9.0 PL software on the basis of the General Linear Model (GLM). The following statistical model was used:

\[ Y_{ijkl} = \mu + G_i + s_j + LAC_k + CS_l + \beta (x_l - A_l) + e_{ijkl} \]

where:
- \( Y_{ijkl} \) – analyzed trait;
- \( \mu \) – overall mean;
- \( G_i \) – fixed effect of IGF1R genotype: IGF1R/HindIII (1,…3) or IGF1R/Mph1103I (1, 2);
- \( s_j \) – random effect of sire \((j = 1, \ldots 97)\);
- \( LAC_k \) – fixed effect of lactation \((k = 1, 2)\);
- \( CS_l \) – fixed effect of calving season \((l = 1, 4)\);
- \( \beta \) – linear regression coefficient of calving age;
- \( x_l \) – calving age of a cow;
- \( A_l \) – mean calving age;
- \( e_{ijkl} \) – random error

RESULTS AND DISCUSSION

In the analysed herd of Holstein-Friesian cows of Black-and-White strain the highest frequency for the IGF1R/HindIII polymorphism was found for the BB genotype (0.4946), a similar one was in the case of the AB genotype (0.4565), whereas the lowest one was for the AA genotype (0.0489). The allele frequency was as follows: allele A – 0.2772, allele B – 0.7228.

Restriction analysis of the 231 bp fragment using the HindIII restriction enzyme allowed identification of two alleles (A and B) determining the occurrence of three genotypes: AA (188 and 43 bp), AB (231 bp, 188 bp and 43 bp) and BB (231 bp; not digested).

In the case of the IGF1R/Mph1103I polymorphism, two genotypes were identified: AB (231 bp, 164 bp and 67 bp) and BB (231 bp – no digestion of the PCR product), whose occurrence was determined by two alleles: allele A and allele B. Individuals with the AA genotype (164 bp and 67 bp) were not found. The genotyping results of both polymorphisms are presented in Table 1.
The BB genotype occurred most frequently (0.9402), whereas the AB genotype was less frequent (0.0598). The individuals with the AA genotype were not found. In the examined herd, the frequency of allele B was 0.9701, whereas that of the rare allele A was 0.0299.

Table 1. The number and frequency of *IGF1R/Hinfl* and *IGF1R/Mph1103I* genotypes and alleles of cows under study

<table>
<thead>
<tr>
<th></th>
<th><em>IGF1R/Hinfl</em> Genotypes</th>
<th>Total</th>
<th>Allele</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>IGF1R/Hinfl</em> genotyp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>AB</td>
<td>BB</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>9</td>
<td>84</td>
<td>91</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.0489</td>
<td>0.4565</td>
<td>0.4946</td>
</tr>
<tr>
<td></td>
<td><em>IGF1R/Mph1103I</em> Genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>AB</td>
<td>BB</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>0</td>
<td>11</td>
<td>173</td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td>0.0000</td>
<td>0.0598</td>
<td>0.9402</td>
</tr>
</tbody>
</table>

For each of the polymorphisms, the statistical analysis was performed and the results are presented in Tables 2 and 3. The association between the milk traits and presented polymorphic sites has not been studied in the available literature.

In the examined herd of cows, in the case of the *IGF1R/Hinfl* polymorphism, a statistically significant (P≤0.01) favourable effect of the BB genotype on the milk yield (+768 kg) was found in comparison with the AB genotype.

The analysis of the milk fat yield revealed the highest value of this trait in animals with the homozygous AA genotype (322 kg), although the difference was not statistically significant in comparison with other genotypes. Individuals with the BB genotype were characterized by significantly higher (P≤0.05) milk fat yield (+23 kg) compared to cows with the heterozygous AB genotype.

In the case of the milk fat content, the highest value of this trait was observed in individuals with the AA genotype (4.26%), whereas the lowest value was found in individuals with the BB genotype (4.10%). However, the differences were not statistically significant.

The milk of cows with the AA genotype was characterised by the highest yield and percentage content of protein compared to milk of cows with the remaining genotypes, which was not proved statistically. Statistically significant differences (P≤0.01) were found only in the milk protein content between the individuals with the AB (232 kg) and BB (255 kg) genotypes.
The mean values of the selected milk performance traits of cows depending on the \textit{IGF1R/Mph}1103I genotype are presented in Table 3. Since the occurrence of the AA genotype was not found in the analyzed herd of Holstein-Friesian cows, this genotype was not included in the statistical analysis.

Table 2. Mean values of the examined production traits of cows corrected for calving age with the \textit{IGF1R/HinfI} gene variants for the Holstein-Friesian cows of Black-and-White strain (standard errors in parenthesis)

<table>
<thead>
<tr>
<th>Polymorphism Polimorfizm</th>
<th>Genotype Genotyp</th>
<th>n</th>
<th>Milk yield, kg Wydajność mleka, kg</th>
<th>Fat – Tłuszcz</th>
<th>Protein – Białko</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>kg</td>
<td>%</td>
<td>kg</td>
</tr>
<tr>
<td>\textit{IGF1R/HinfI}</td>
<td>\textit{AA}</td>
<td>9</td>
<td>7516 (877.23)</td>
<td>322 (50.55)</td>
<td>4.26 (0.36)</td>
</tr>
<tr>
<td></td>
<td>\textit{AB}</td>
<td>84</td>
<td>6852 (198.22)</td>
<td>288 (8.37)</td>
<td>4.22 (0.06)</td>
</tr>
<tr>
<td></td>
<td>\textit{BB}</td>
<td>91</td>
<td>7620 (167.94)</td>
<td>311 (7.69)</td>
<td>4.10 (0.06)</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>184</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means within columns bearing the same superscripts differ significantly at: A – P ≤ 0.01, a – P ≤ 0.05.

No significant effect of the \textit{IGF1R/Mph}1103I polymorphic site on the yield of milk, milk fat and protein was found. Statistically significant differences (P≤0.05) were observed only for the percentage content of milk fat and protein. Milk of cows with the BB genotype was characterized by a higher percentage content of protein (+0.19) and fat (+0.39) compared to milk of cows with the \textit{AB} genotype.

Many examples of polymorphisms occurring in the bovine insulin-like growth factor I receptor gene have been described but there is little information on the effect of these changes in the DNA on the milk and meat traits. Moody et al. [1996] described for the first time a polymorphic site located in intron 12 recognized by the \textit{TaqI} enzyme. These authors found that the mutation is very rare and occurs only in \textit{Bos indicus} cattle, whereas it was not found in \textit{Bos taurus}. Only a few studies on this polymorphism have been published. According to the studies by Akis et al. [2010], the \textit{IGF1R/TaqI} polymorphism did not affect the improvement of the milk and meat traits in cattle. Also Curi et al. [2005] showed that the above-mentioned polymorphism did not have any significant effect on the body weight gains, carcass composition and quality.
Table 3. Mean values of the examined production traits of cows corrected for calving age with the IGF1R/Mph1103I gene variants for the Holstein-Friesian cows of Black-and-White strain (standard errors in parenthesis)

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype</th>
<th>Milk yield, kg</th>
<th>Fat – Tłuszcz</th>
<th>Protein – Białko</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wydajność mleka, kg</td>
<td>kg</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>7363 (541.41)</td>
<td>279 (20.83)</td>
<td>3.81a (0.23)</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>11 11 7252 (138.91)</td>
<td>301 (6.19)</td>
<td>4.20a (0.04)</td>
</tr>
<tr>
<td>IGF1R/Mph1103I</td>
<td>BB</td>
<td>173</td>
<td>279 (20.83)</td>
<td>3.81a (0.23)</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>184</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means within columns bearing the same superscripts differ significantly at: A – P ≤ 0.01, a – P ≤ 0.05. Średnie w kolumnach oznaczone tymi samymi literami różnicą się istotnie: A – P ≤ 0.01, a – P ≤ 0.05.

Insulin-like growth factor I (IGF-I) is one of the elements of the somatotropic axis. Due to the significant function during the postnatal period and the ontogenesis it is a good candidate gene for genetic marker. Its function is strictly associated with the presence of the specific receptor whose polymorphism is analysed in the present study. The local expression of the genes coding for IGF1 and its receptor in the mammary gland is regulated physiologically and their protein products play a key role in the development and functioning of this gland [Plath-Gabler et al. 2001]. Since the time when it was observed that IGF-I is mainly responsible for the growth process during the postnatal period, it has also been found that any permanent effects of the changes in the level of IGF1 and IGF1R gene expression in the skeletal muscles of the foetus during the pre- and neonatal period can also mediate the subsequent different increase in muscle mass in the postnatal period [Micke et al. 2011]. Therefore, the research in this field should be continued on a higher number of individuals, not only of dairy but also of beef breeds.

CONCLUSIONS

Two original PCR-RFLP protocols (for IGF1R/HinfI and IGF1R/Mph1103I polymorphisms) that were used to prove the occurrence of two mutations (rs41960620 and rs41960621) in intron 4 of the insulin-like growth factor I receptor gene (IGF1R) in domestic cattle (Bos taurus) were devised. For the IGF1R/HinfI polymorphism, different frequencies of genotypes and alleles were recorded. Allele B dominated, whereas no individuals with the AA genotype were found for the IGF1R/Mph1103I. A high frequency of allele B (0.82–0.98) was observed. The IGF1R/HinfI polymorphism, due to the varied
frequencies of alleles and genotypes, can be a valuable material in the association studies conducted on larger populations. A high frequency of allele $B$ and absence of cows with the $AA$ genotype may limit the usefulness of the $IGF1R/Mph1103I$ polymorphism for the association studies. Due to the fact that there is no available literature on the subject under discussion, it was not possible to carry out a comparative analysis of the obtained results, which indicates the need for further investigation into $IGF1R$ gene polymorphism in the context of its practical application to the cattle farming.

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**REFERENCES**


**ZWIĄZEK POMIĘDZY DWOMA MIEJSCAMI POLIMORFICZNYMI W INTRONIE 4 GENU KODUJĄCEGO RECEPTOR INSULINOPODOBNEGO CZYNNIKA WZROSTU TYPU 1 (IGF1R/Mph1103I ORAZ IGF1R/HinfI) A CECHAMI UŻYTKOWOŚCI MLECZNEJ BYDŁA POLSKIEGO HOLSZTYŃSKO-FRYZYSKIEGO**

**Streszczenie.** Celem niniejszej pracy było określenie frekwencji dwóch wariantów polimorficznych zlokalizowanych w intronie 4 genu kodującego receptor insulinoподобnego czynnika wzrostu typu 1 (*IGF1R*) w badanym stadem 184 krów holsztyńskiego-fryzyjskiego oraz poszukiwanie związku pomiędzy tymi polimorfizmami a wybranymi cechami użytkowości mlecznej. Polimorfizm genu *IGF1R* identyfikowano metodą PCR-RFLP z zastosowaniem enzymów restrykcyjnych: *HinfI* oraz *Mph1103I*. W układzie *IGF1R/HinfI* stwierdzono najwyższą frekwencję genotypu $BB$ (0,49), zbliżoną w przypadku genotypu $AB$ (0,45) a najniższą dla genotypu $AA$ – 0,04. Frekwencja alleli kształtowała się następująco: allele $A$ – 0,28, allele $B$ – 0,72. Analiza
statystyczna wykazała, że analizowany polimorfizm wpływał istotnie na wydajność mleczną, wydajność białka (P≤0,01) oraz tłuszczu (P≤0,05) w mleku, faworyzując genotyp BB. W układzie IGF1R/Mph1103I odnotowano brak osobników o genotypie AA. Stwierdzono wysoką frekwencję allelu B (0,94). Nie wykazano istotnego wpływu polimorficznego IGF1R/Mph1103I na wydajność mleka, tłuszczu i białka. Statystycznie istotne różnice (P≤0,05) zaobserwowano jedynie w % zawartości tłuszczu i białka w mleku ze wskazaniem na pozytywny wpływ genotypu BB.

Słowa kluczowe: IGF1R, polimorfizm, wydajność mleczna, QTL

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