

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 ($P \leq 0.01$) and milk fat yield ($P \leq 0.05$), 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 ($P \leq 0.05$) 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

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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. *MasterPureTM 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'– CTGGATATGTCCGCCTTAGC – 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, IBB PAN, 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_a) 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 *HinfI* 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 *HinfI* 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/HinfI* 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:

$$Y_{ijkl} = \mu + G_i + s_j + LACK + CS_l + \beta (x_l - A_l) + e_{ijkl}$$

where:

Y_{ijkl} – analyzed trait; μ – overall mean; G_i – fixed effect of *IGF1R* genotype: *IGF1R/HinfI* (1,...3) or *IGF1R/Mph1103I* (1, 2); s_j – random effect of sire ($j = 1, \dots, 97$); *LACK* – fixed effect of lactation ($k = 1, 2$); CS_l – fixed effect of calving season ($l = 1, 4$); β – linear regression coefficient of calving age; x_l – calving age of a cow; A_l – mean calving age; e_{ijklm} – random error

RESULTS AND DISCUSSION

In the analysed herd of Holstein-Friesian cows of Black-and-White strain the highest frequency for the *IGF1R/HinfI* 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 *HinfI* 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/HinfI* and *IGF1R/Mph1103I* genotypes and alleles of cows under study

Tabela 1. Częstość występowania genotypów i alleli dla układu *IGF1R/HinfI* i *IGF1R/Mph1103I*

	<i>IGF1R/HinfI</i> genotypes <i>IGF1R/HinfI</i> genotyp			Total Razem	Allele Allele	
	<i>AA</i>	<i>AB</i>	<i>BB</i>		<i>A</i>	<i>B</i>
n	9	84	91	184		
Frequency Frekwencja	0.0489	0.4565	0.4946	1.0000	0.2772	0.7228

	<i>IGF1R/Mph1103I</i> Genotypes <i>IGF1R/Mph1103I</i> Genotyp			Total Razem	Allele Allele	
	<i>AA</i>	<i>AB</i>	<i>BB</i>		<i>A</i>	<i>B</i>
n	0	11	173	184		
Frequency Frekwencja	0.0000	0.0598	0.9402	1.0000	0.0299	0.9701

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/HinfI* polymorphism, a statistically significant ($P \leq 0.01$) 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 ($P \leq 0.05$) 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 ($P \leq 0.01$) 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 *IGF1R/Mph1103I* 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 *IGF1R/HinfI* gene variants for the Holstein-Friesian cows of Black-and-White strain (standard errors in parenthesis)

Tabela 2. Przeciętne wartości średnie badanych cech użytkowych krów skorygowane ze względu na wiek wycielenia krów z wariantami genu *IGF1R/HinfI* dla krów rasy holsztyńsko-fryzyjskiej odmiany czarno-białej (w nawiasie błędy standardowe)

Polymorphism Polimorfizm	Genotype Genotyp	n	Milk yield, kg Wydajność mleka, kg	Fat – Tłuszcz kg	Protein – białko kg		
<i>IGF1R/HinfI</i>	<i>AA</i>	9	7516	322	4.26	257	3.50
			877.23	50.55	0.36	29.66	0.15
	<i>AB</i>	84	6852 ^A	288 ^a	4.22	232 ^A	3.40
			198.22	8.37	0.06	6.66	0.03
	<i>BB</i>	91	7620 ^A	311 ^a	4.10	255 ^A	3.37
			167.94	7.69	0.06	4.85	0.04
	total ogółem	184					

Means within columns bearing the same superscripts differ significantly at: A – $P \leq 0.01$, a – $P \leq 0.05$.
Średnie w kolumnach oznaczone tymi samymi literami różnią się istotnie: A – $P \leq 0,01$, a – $P \leq 0,05$.

No significant effect of the *IGF1R/Mph1103I* polymorphic site on the yield of milk, milk fat and protein was found. Statistically significant differences ($P \leq 0.05$) 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 *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 *TaqI* enzyme. These authors found that the mutation is very rare and occurs only in *Bos indicus* cattle, whereas it was not found in *Bos taurus*. Only a few studies on this polymorphism have been published. According to the studies by Akis et al. [2010], the *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)

Tabela 3. Przeciętne wartości średnie badanych cech użytkowych krów skorygowane ze względu na wiek wycielenia krów z wariantami genu *IGF1R* dla krów rasy hol-sztyńsko-fryzyjskiej odmiany czarno-białej (w nawiasie błędy standardowe)

Polymorphism Polimorfizm	Genotype Genotyp	n	Milk yield, kg Wydajność mleka, kg	Fat – Tłuszcz kg	Protein – białko kg
<i>IGF1R/Mph1103I</i>	<i>AA</i>	–	–	–	–
	<i>AB</i>	11	7363 541.41	279 20.83	3.81 ^a 0.23
	<i>BB</i>	173	7252 138.91	301 6.19	4.20 ^a 0.04
	total ogółem	184			

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

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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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-FRYZYJSKIEGO

Streszczenie. Celem niniejszej pracy było określenie frekwencji dwóch wariantów polimorficznych zlokalizowanych w intronie 4. genu kodującego receptor insulinopodobnego czynnika wzrostu typu 1 (*IGF1R*) w badanym stadzie 184 krów rasy holsztyńsko-fryzyjskiej 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: allel *A* – 0,28, allel *B* – 0,72. Analiza

statystyczna wykazała, że analizowany polimorfizm wpływał istotnie na wydajność mleczną, wydajność białka ($P \leq 0,01$) oraz tłuszczu ($P \leq 0,05$) 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 ($P \leq 0,05$) 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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