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Genetic polymorphisms of *FAM13A1*, *OPN*, *LAP3*, and *HCAP-G* genes in Jersey cattle

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Abstract: The aim of this experiment was to estimate possible associations between *FAM13A1*, *OPN*, *LAP3*, and *HCAP-G* genotypes and some milk performance traits, like daily milk yields (kg), protein content (%), and fat content (%). The study included 181 Jersey cows. The polymerase chain reaction-restriction fragment length polymorphism and artificial constructed restriction sites methods were used to identify genotypes. The minor allele frequencies were as follow: F*AM13A1 G/A* 0.34, *FAM13A1 A/C* 0.44, *OPN T/C* 0.22, *OPN G/T* 0.38, *LAP3* 0.07, and *HCAP-G* 0.49. No associations were observed between the single-nucleotide polymorphisms in analyzed loci and the traits in this study; however, the findings showed some tendencies towards reaching lower or higher values of a given trait by an individual of a given genotype.

Key words: Jersey cattle, milk performance traits, BTA6

1. Introduction

Studies by numerous authors indicate that many bovine chromosomes (BTAs), including BTA3, 6, 9, 14, 20, and 23, in particular, bear clusters of quantitative trait loci (QTL) of different milk performance traits. In numerous reports the studies concentrated on BTA6 within 2 regions, in which clusters of genes associated with milk performance traits are located (1). These regions are the fragments in the middle part of BTA6, close to the BM143 marker, and the region close to the casein genes complex (2,3). Previous literature suggests that genes located near BM143 affect 5 milk performance traits, including milk yield (kg), milk protein and fat yield (kg), and milk protein and fat content (%) (1). The region encompassing these genes extends from the *FAM13A* (family with sequence similarity 13, member A1) to the *PPARGC1A* (peroxisome proliferator-activated receptor-γ coactivator-1α) genes and spans approximately 6.2 cM. In this range, there are genes whose effects on the performance traits of cattle have already been examined, such as *OPN* (osteopontin), and genes that may potentially influence these traits, such as *FAM13A1*, *LAP3* (leucine aminopeptidase 3), and *HCAP-G* (condensin subunit 3) (4–6).

Osteopontin (OPN) is a phosphorylated glycoprotein that plays a role in different processes in the organism, for example in cell adhesion, chemotaxis, cell signaling, and the regulation of growth and development of the fetus, as well as in the initiation and maintenance of pregnancy (7). The presence of OPN in milk and its high level of expression in mammary epithelial cells may cause proliferation and differentiation of the mammary gland (8). Despite fulfilling so many functions, OPN is treated by most authors as a positional candidate gene for milk-performance traits harboring the QTL region on BTA6 (9,10). Other studied genes do not play such an important role in the organism and, therefore, are considered first of all as positional candidate genes.

It was suggested that the *FAM13A1*, *LAP3*, and *HCAP-G* genes may be associated with milk-performance traits such as milk yield and milk composition, mainly due to their location in the analyzed region and due to the fact that the expression levels of these genes were found to be different in the mammary gland (4,11–13). Preliminary assessments of the association of these genes with milk-performance traits were reported (5,11–13). For instance, the *LAP3* gene may affect milk fat and protein yield, the *FAM13A1* and *LAP3* genes may affect the change in milk protein content, and the *LAP3* gene has been associated with milk yield (5,11–13).

On the basis of the above-mentioned data, this research aimed at determining the allele and genotype frequencies of the single-nucleotide polymorphisms (SNPs) in the *FAM13A1*, *OPN*, *LAP3*, and *HCAP-G* genes in a herd of Jersey cows, as well as establishing possible associations between the genotypes and the milk-performance traits of the examined herd of cattle.

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2. Materials and methods

Animal material included 181 dairy Jersey cows kept in the Wielkopolska region of Poland. The cows came from 17 sires. All studied animals were kept in identical environmental conditions. The cows were fed a standard diet and were kept on pasture during spring and summer time. The animals were milked twice daily using a mechanical milking machine. The herd's milk yield was evaluated by the A4 method, consistent with the recommendations of the International Committee for Animal Recording.

The DNA used in the analysis was isolated from the peripheral blood from the jugular vein and collected into vacuum test tubes containing K_3-EDTA as an anticoagulant. The DNA isolation was performed using the Master Pure™ kit (Epicentre®) according to the manufacturer's instructions.

Genotype analyses were performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and artificial constructed restriction sites methods. PCR primers were designed mainly based on sequences available in GenBank (*FAM13A1* - AJ510139, *OPN* - AJ871176, *LAP3* - AY744570, *HCAP-G* - AY744569). In the case of *FAM13A1* (exon 12) and *OPN* (intron 4), genotypes were identified according to Cohen et al. (11) and Leonard et al. (14), respectively. Table 1 shows the used primer sequences, restriction enzymes, PCR product sizes, and size of fragments after restriction endonuclease digestion.

Obtained genotyping results were statistically analyzed. PopGene version 1.32 (15) was used for estimation of allele frequencies and Hardy–Weinberg equilibrium of genotype frequencies was calculated. Linkage disequilibrium (LD) measures (r^2) were obtained using Haploview software (http://www.broadinstitute.org). LD blocks were determined using the Gabriel criteria (16).

Further statistical analysis of associations between the SNPs and milk performance traits [i.e. daily milk yield (kg), fat content (%), and protein content (%)] was performed according to the general linear model (GLM) procedure using Statistica Software version 7.1. (17). The mixed multiple-factor model of the GLM platform was used. The sire effect was not included in the statistical model because preliminary statistical analysis showed that the sire effect was not significant.

The following linear model was applied:

$$y_{ijklm} = \mu + a_i + b_j + c_k + d_l + f_m(a_i) + e_{ijklm},$$

where μ = trait mean; a_i = genotype effect (i = 1, 2, 3); b_j = lactation number effect (j = 1, 2, 3, 4, 5); c_k = lactation season effect (k = 1, 2, 3, 4); d_l = lactation month effect (l = 1, 2, 3, ..., 14); $f_m(a_i)$ = cow effect, a random factor nested within genotypes (m = 1, 2, ..., 181); and e_{ijklm} = random error.

Differences between average values of the traits were analyzed using Duncan's multiple range test.

3. Results

The frequencies of genotypes and alleles obtained for each of the examined SNPs are presented in Table 2. In most analyzed loci, the occurrence of all 3 possible genotypes determined by the presence of 2 alleles was found. Only in the case of *OPN G/T* and *LAP3 T/C* was the occurrence of only 2 out of the 3 possible genotypes observed. A very low frequency of the recessive allele was found for the *LAP3 T/C* and *OPN T/C* polymorphisms. This resulted in the presence of only 2 out of the 3 possible genotypes in the case of *LAP3 T/C*. On the other hand, the occurrence of all 3 possible genotypes was shown in the case of *OPN T/C*;

Table 1. Conditions for PCR-RFLP of the analyzed polymorphisms (mismatched nucleotides are underlined).

SNP	Primers	PCR	T	RE	D
FAM13A1 Intron 9, G/A	**F:**CCCCGCTTCTACGTTACGGGCAGA **R:**GATATACTTACAACCTAGCTAAA**C**A	199	50 °C	*Nde*I	*G*:199 *A*: 172, 27
FAM13A1 Exon 12, C/A	**F:**CACGCCCAAATCTTTTCTCT **R:**GGGCTCATCACAGAATCACA	265	58 °C	*Ava*II	*C*: 265 *A*: 216, 49
OPN Intron 4, T/C	**F:**GCAAATCAGAAGTGTGATAGAC **R:**CCAAGCCAAACGTATGAGTT	290	56 °C	*Bsr*I	*T*: 290 *C*: 200, 90
OPN Exon 7, G/T	**F:**ACCCTGCTTT**A**ATGTATCCTTTAC **R:**GTCAGGAAAATTCCAAACTCAGCC	204	52 °C	*Tai*I	*G*: 178, 26 *T*: 204
LAP3 Exon 12, T/C	**F:**GACAGGTTATAGATTGCCAACT**G**GC **R:**TTTAGATTTGAAAATGCAAAAACCA	250	54 °C	*Hae*III	*T*: 250 *C*: 226, 24
HCAP-G Intron (number is not known) A/C	**F**:TCCAAAATCAATAACAGCGTC**G**GC **R**:GGAGGAGGGCATGGCATTCTT	188	59 °C	*Hae*III	*A*: 141, 47 *C*: 118, 47, 23

PCR: PCR product size (bp); T: annealing temperature; RE: restriction endonuclease; D: digestion product size (bp).

Table 2. Genotype and allele frequencies of the analyzed SNPs.

SNP	Genotype	Frequency	Allele	Frequency
FAM13A1	*GG*	0.326	*G*	0.66
	GA	0.672	*A*	0.34
	AA	0.002		
FAM13A1	*AA*	0.203	*A*	0.56
	AC	0.723	*C*	0.44
	CC	0.074		
OPN	*TT*	0.011	*T*	0.22
	TC	0.411	*C*	0.78
	CC	0.578		
OPN	*GT*	0.755	*G*	0.38
	TT	0.245	*T*	0.62
LAP3	*TT*	0.850	*T*	0.93
	TC	0.150	*C*	0.07
HCAP-G	*AA*	0.006	*A*	0.49
	AC	0.976	*C*	0.51
	CC	0.018		

however, only 3 *TT* homozygous individuals were found in the whole examined herd.

All analyzed SNPs are located in chromosome 6 in the region extending for approximately 400 kb. Of these SNPs, 2 are located in the *FAM13A1* gene and 2 in the *OPN* gene. The SNP in the *LAP3* gene was not included in the LD analysis, since the Hardy–Weinberg equilibrium was retained in this case. LD (r^2) between the analyzed pairs of SNPs was as follow: r^2 for *FAM13A1 G/A* and *FAM13A1 A*/C SNPs was 0.27, and r^2 for SNPs *OPN T/C* and *OPN G/T* was 0.08. For most SNP pairs, the calculated r^2 coefficient has low values, which may indicate the small effect between loci. Only in the case of the *OPN G/T* and *HCAP-G A/C* pair was r^2 0.54, which indicates a moderate correlation between these SNPs.

The mean values of the analyzed milk performance traits with regard to the individual genotypes of examined genes are presented in Table 3. The genotypes for which there were fewer than 20 samples for a given polymorphic variant were not included in the table or the statistical analysis. No significant association between the SNPs in the examined genes and the analyzed traits was found. However, it is worth mentioning that some associations between different genotypes of the examined SNPs and the mean values of the analyzed milk-performance traits were observed.

4. Discussion

QTLs located in the proximity of the middle of chromosome 6 (BTA6), affecting the milk protein content, were discovered for the first time by Georges et al. (18) in a population of Holstein cattle in the United States. The same QTLs were also discovered in other populations of Holstein cattle as well as in breeds such as Finnish Ayrshire (19) and Norwegian cattle (20). Ron et al. (13) showed that the region containing the analyzed QTLs was located approximately 4 cM from the BM143 microsatellite, whereas Olsen et al. (20), using physical mapping, combined linkage and LD mapping and determined the location of these QTLs. They were located within a 420-kb region lying between the *ABCG2* and *LAP3* genes (12).

In this study, no correlation was found between SNPs and analyzed milk-production traits, but some trends were observed, which could be verified in future research with a greater number of animals. In the case of daily milk, the yield tended to achieve higher values than the average for the herd. For next analyzed trait, the fat content of milk, a negative association was observed; that is, a lower value was achieved than the value of the average trait for most of the analyzed SNPs. In the case of the other analyzed milk-production trait, the protein content of milk, a higher value for this trait was noted for most of the analyzed SNPs than the average.

Association between SNPs in the genes located in the analyzed BTA6 fragment and performance traits of cattle has also been studied by various other authors. Although no significant differences in the mean values of the analyzed traits between the individuals with different genotypes of the analyzed loci were found in the presented study, other authors showed an effect of polymorphic variants on milk-performance traits. In the study by Cohen et al. (11), the negative effect of allele *C* on milk yield and the positive effects of this allele on the remaining examined traits were shown for *FAM13A1 C/A*; however, only the effect of this allele on milk protein content was significant. In the same study, it was shown for the *FAM13A1 G/A* SNP that allele *A* also negatively affects milk yield, whereas it positively influences milk fat and protein content. In the case of the SNP in *HCAP-G A/C*, no association between this polymorphism and the examined milk performance traits was found (12). In the study by Cohen-Zinder et al. (4), a significant effect of allele *C* of *LAP3 T/C* on milk fat and protein yield was shown. However, in the same study a significant negative influence of allele *T* on the milk yield was found for the *OPN G/T* SNP. In the case of *OPN T/C* polymorphism, it was found that allele *C* positively affected milk fat and protein content and negatively influenced milk yield; however, this effect was significant in one study (14) and nonsignificant in another (21).

Three of the analyzed SNPs are located in exons: *FAM13A1 C/A* in exon 12, *OPN G/T* in exon 7, and *LAP3 T/C* in exon 12. In the case of *FAM13A1*, it was shown that the C/A exchange of nucleotides leads to a nonsynonymous amino acid substitution (Ile/Leu). Both amino acids are nonpolar. Therefore, this exchange does not result in

Table 3. Means and standard deviations of milk production traits.

SNP	Genotype	N	Milk kg	Fat %	Protein %
FAM13A1 A/C	*AA*	283	15.52 ± 4.52	5.96 ± 0.98	4.09 ± 0.52
	AC	1009	15.07 ± 4.75	5.80 ± 1.03	4.11 ± 0.56
	CC	103	15.20 ± 4.73	5.69 ± 1.16	4.11 ± 0.55
	Total	1395	15.17 ± 4.70	5.82 ± 1.04	4.11 ± 0.55
FAM13A1 A/G	*GG*	455	15.15 ± 4.85	5.78 ± 1.04	4.07 ± 0.51
	GA	937	15.20 ± 4.63	5.85 ± 1.04	4.15 ± 1.19
	Total	1392	15.16 ± 4.71	5.83 ± 1.04	4.11 ± 0.56
OPN T/C	*TC*	573	15.4 3± 4.78	5.69 ± 0.99	4.06 ± 0.54
	CC	807	15.04 ± 4.65	5.92 ± 1.06	4.18 ± 1.25
	Total	1395	15.17 ± 4.70	5.82 ± 1.04	4.11 ± 0.55
OPN G/T	*GT*	1053	15.11 ± 4.76	5.83 ± 1.01	4.12 ± 0.55
	TT	342	15.36 ± 4.52	5.79 ± 1.06	4.16±1.81
	Total	1395	15.17 ± 4.70	5.82 ± 1.04	4.11 ± 0.55
LAP3	*TT*	1186	15.07 ± 4.74	5.85 ± 1.06	4.12 ± 0.57
	TC	209	15.78 ± 4.56	5.78 ±0 .82	4.00 ± 0.50
	Total	1395	15.17 ± 4.70	5.82 ± 1.04	4.11 ± 0.55
HCAP-G	*AC*	1362	15.20 ± 4.69	5.80 ± 1.02	4.10 ±0 .54
	CC	24	12.99 ± 5.21	6.64 ± 1.32	4.53 ± 0.76
	Total	1386	15.16 ± 4.71	5.82 ± 1.04	4.11 ± 0.56

N: number of test milk yields; milk kg: daily milk yield; fat %: fat content; protein %: protein content.

considerable changes in the protein conformation (11). In the case of *LAP3* and the T/C nucleotide exchange, the Ala synonymous amino acid substitution occurs. Therefore, this SNP is not visible in the posttranslational structure of the LAP3 polypeptide (GenBank CAI44744). On the other hand, in the case of *OPN G/T*, the SNP is indeed located in the exon, but in its noncoding part (3'UTR), which is not translated (9). Despite the above-mentioned SNPs being located in exons, they do not significantly affect the structure of their product. Therefore, all the analyzed SNPs, both mapped to introns and exons, can be regarded as performance trait markers during future research.

It can be concluded that the present study does not show statistically significant differences between individual genotypes of Jersey cattle in respect to the aforementioned traits. However, due to this analysis, it was possible to show some tendencies towards reaching lower or higher values of a given trait by an individual of a given genotype. For this reason, it seems justified to conduct further research on a larger group of animals or different breeds of cattle in order to verify the results of the present study.

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