

## The association between microsatellite *BM6438* and milk performance traits in Polish Holstein-Friesian cattle

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**ABSTRACT:** The objective of this study was to verify the hypothesis postulating the location of QTL linked marker for milk performance traits in the proximal section of chromosome BTA1 by analyzing the microsatellite *BM6438* alleles from heterozygous sires and the milk performance traits of the investigated cattle. The experiment covered 484 Polish Holstein-Friesian primiparous cows, the progeny of three unrelated sires, including 317 cows – the progeny of two sires with 256/268 genotype and 167 cows – the progeny of a sire with 258/268 genotype. *BM6438* polymorphism was determined by PCR and polyacrylamide gel electrophoresis. Three alleles (256, 258 and 268), forming 6 genotype groups, were identified among the studied cattle. In the progeny of sires with 256/268 genotype, statistically significant differences were found out in protein yield (the progeny of 2 sires) and in milk yield (the progeny of 1 sire). In both cases, the highest values of performance traits were recorded in 268/268 genotype groups, and the lowest in half-sib groups with 256/258 and 258/268 genotypes. The analysis covering the progeny of two sires with 256/268 genotype confirmed the regularities noted in individual families. 268/268 homozygotes had a highly significantly higher ( $P \leq 0.01$ ) protein yield than the remaining genotype groups. Statistically significant differences were also reported in the values of milk yield ( $P \leq 0.05$ ).

**Keywords:** cattle; Polish Holstein-Friesian breed; *BM6438* polymorphism; milk performance traits

The QTL linked markers for milk performance traits were found out in all bovine autosomes (Georges et al., 1995; Mosig et al., 2001; Nadesalingam et al., 2001; Rodriguez-Zas et al., 2002; Ashwell et al., 2004; Khatkar et al., 2004; Ron et al., 2004; Schrooten et al., 2004; Polineni et al., 2006; Bagnato et al., 2008; Lund et al., 2008). Only a small number of quantitative trait nucleotides (QTN) or causative mutations (Winter et al., 2002; Blott et al., 2003; Cohen-Zinder et al., 2005; Schnabel et al., 2005) have been identified for economically important cattle QTLs.

To date, various authors have postulated the location of milk performance QTLs in different regions

of chromosome BTA1. Georges et al. (1995) were the first to demonstrate that the proximal segment of chromosome 1 (BTA1) between *TGLA49* and *TGLA57* affected milk and protein yield. Subsequent investigations recognized a QTL region flanked by *AGLA17* and *CA095* (0–23.9 cM) associated with milk yield (Nadesalingam et al., 2001; Khatkar et al., 2004), protein yield (Zhang et al., 1998) as well as protein and fat content (Nadesalingam et al., 2001). In the light of present knowledge, no candidate genes responsible for milk performance traits have been determined on chromosome BTA1.

In a new assembly of the *Bos taurus* genome in Ensembl of Btau 4.0, the *BM6438* marker is as-

signed on BTA1 at the position of 1832998–1833255 ([http://www.ensembl.org/Bos\\_taurus/Info/Index](http://www.ensembl.org/Bos_taurus/Info/Index)). However, in cattle QTLdb (<http://www.animalgenome.org/cgi-bin/QTLdb/BT/index>), *BM6438* is localized at 1.78 cM of BTA1 (AnimalQTLdb:7127). This region has been identified as QTL region (Gene ID: 100308331; SSSA: Social separation – Standing alert) affecting cattle temperament (Gutiérrez-Gil et al., 2008). The above statement justifies the need for further research into the selection of QTL-linked markers that are most useful in breeding practice. In a previous study, Czarnik et al. (2005) determined the changes in the allele frequencies of *BM6438* during the improvement process of Polish dairy cattle.

The objective of this study was to investigate the relationship between the putative QTL-linked microsatellite marker *BM6438* and performance traits of the Polish Holstein-Friesian cattle.

## MATERIAL AND METHODS

The experiment covered 484 Polish Holstein-Friesian primiparous cows, the progeny of three sires, including 317 cows – the progeny of two sires with the 256/268 genotype (sire I and II) and 167 cows – the progeny of a sire with the 258/268 genotype (sire III). To find heterozygotic sires having sufficient numbers of progeny, 20 sires were screened in preliminary studies (data not shown). Three sires included in the analyses were not related to each other, more than average inbreeding coefficient estimated for the Polish Holstein-Friesian breed.

The database of milk performance traits comprised milk yield, fat yield, protein yield, percentage content of fat and protein in the milk of cows of the same age, recorded during the first 305-day lactation. To reduce the impact of environmental factors on the analysed traits, cows originated from herds kept in very similar feeding and housing systems and welfare conditions. The investigated half-sib heifers were calved during the year evenly and were kept indoors.

The polymorphism of microsatellite marker *BM6438* was determined by PCR and polyacrylamide gel electrophoresis. Genomic DNA was isolated from semen or peripheral blood samples using a MasterPure DNA Purification Kit (Epicenter, Madison, USA). PCR was carried out in a final volume of 25 µl, containing 1 µl of genomic DNA (100 ng/µl), 2.0 µl of 25mM MgCl<sub>2</sub>, 1.25 µl of 20 ×

PCR buffer (400mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.0M TRIS-HCl pH 9.0), 0.2 µl of each primer (100µM), 1.5 µl of 2.0mM dNTPs, 0.5 units of *Tfl* DNA polymerase and 3.0 µl of enhancer (Epicenter, Madison, USA). The primer sequences of *BM6438* were adopted according to Bishop et al. (1994):

Forward primer: 5' TTG AGC ACA GAC ACA GAC TGG 3' – labelled with IRD 800

Reverse primer: 5' ACT GAA TGC CTC CTT TGT GC 3'

A T3-thermocycler (Biometra, Goettingen, Germany) was used to perform amplification under the following thermal profile: initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C (1 min), annealing temperature touched down from 70°C to 61°C (1 min), elongation 72°C (1 min), with a final extension step of 10 min at 72°C. Subsequently, PCR products were examined using 6% polyacrylamide sequencing gel and visualized in the Genetic Analyzer (LI-COR, Lincoln, USA).

## Statistics

The milk performance traits were analyzed on the basis of 305 days of the first lactation records. The results of variability of milk performance evaluation were compiled taking into account differences in milk yield as well as in fat and protein contents of milk in cows kept in different herds (Czarnik et al., 2007). The relationship between the genetic polymorphism of the marker *BM6438* and the variation in milk performance traits within each of the three families was analyzed by the following linear models using the ANOVA procedures (Statistica 9, Statsoft Inc). The significance of differences between genotype groups was verified by Tukey's HSD test.

Linear models for the co-segregation analysis of sire's paternal allele effects on milk production traits of half-sib progenies (Tables 2–4):

$$Y_{ij} = \mu + G_i + H_j + e_{ij}$$

where:

$Y_{ij}$  = values of traits

$\mu$  = overall mean

$G_i$  = effect of genotype  $i$

$H_j$  = effect of herd  $j$

$e_{ij}$  = random error

Linear models for the joint collective co-segregation analysis of sire's paternal allele effects on

milk production traits of half-sib progenies (Tables 1–5):

$$Y_{ijk} = \mu + G_i + H_j + S_k + e_{ijk}$$

where:

$Y_{ijk}$  = values of traits

$\mu$  = overall mean

$G_i$  = effect of genotype  $i$

$H_j$  = effect of herd  $j$

$S_k$  = effect of sire  $k$

$e_{ijk}$  = random error

## RESULTS

Six genotype groups resulting from a combination of three alleles – 256bp, 258bp and 268bp – were identified in the studied herd of 484 cows (Figure 1).

The joint evaluation of all first lactations of genotyped progeny of all sires (involved sires' effect) showed the statistically significantly ( $P \leq 0.05$ ) higher protein yield of cows with 268/268 genotype than of those with 256/258 and 258/268 genotypes (Table 1).

The relationship between the polymorphism of microsatellite marker *BM6438* and the variation in milk performance traits was next analyzed separately in three half-sib groups. The results are presented in Tables 2–4.

The highest yields of milk and milk components were recorded in half-sib groups with 268/268 genotype, the progeny of 256/268 heterozygotes.

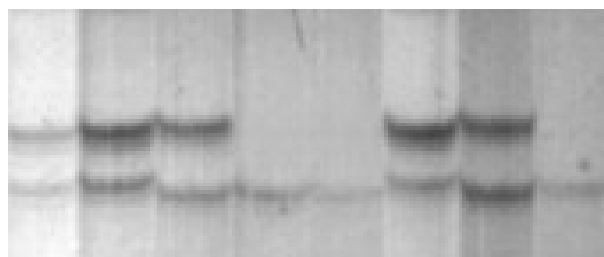


Figure 1. Electrophoregram showing the resolution of three *BM6438* alleles. 256/256 homozygotes – lanes 4, 5 and 8, 256/268 heterozygotes – lanes 3 and 7, 258/268 heterozygotes – lanes 1, 2 and 6

Statistically significant differences were reported in milk yield (the progeny of sire II) and protein yield (the progeny of sires I and II). The 268/268 genotype group of sire II daughters had a higher milk yield in comparison with the half-sibs with homozygous genotype 256/256, while the advantage noted over the half-sibs with 256/268 genotype produced a subthreshold value of  $P = 0.06$ . Positive phenotypic effects of 268/268 genotype were also observed in protein yield: the values recorded in respect of 268/268 homozygotes were higher than those reported for cows of the same age with 256/256 and 256/268 genotype (the progeny of sire II) and 258/268 genotype (the progeny of sire I). Although lower values of protein yield were noted in the two remaining genotype groups of the progeny of sire II – 256/258 and 258/268 – statistically significant differences were not confirmed due to a small group size and a high variability of the analyzed trait ( $P = 0.09$  and  $P = 0.11$ , respectively).

Table 1. The association between *BM6438* polymorphism and milk performance traits in the all tested cows – progenies of 3 heterozygous sires

<i>BM6438</i> genotype	<i>n</i>	Milk yield		Fat				Protein			
		(kg)		yield (kg)		content (%)		yield (kg)		content (%)	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
256/256	94	6721	1468	291	60	4.37	0.54	219	43	3.29	0.26
256/258	89	6478	1032	287	48	4.45	0.54	215 <sup>a</sup>	33	3.34	0.34
258/258	11	6539	1058	300	31	4.65	0.48	221	28	3.40	0.24
256/268	168	6569	1285	291	54	4.46	0.51	218	40	3.33	0.23
258/268	61	6389	1141	291	61	4.55	0.55	210 <sup>b</sup>	38	3.32	0.19
268/268	61	7001	1252	308	49	4.49	0.60	234 <sup>ab</sup>	41	3.36	0.24

<sup>ab</sup>identical indexes (small letters: a or b) designate significant differences between genotype groups at  $P \leq 0.05$

Table 2. The association between *BM6438* polymorphism and milk performance traits in the half-sib progenies of sire I with 256/268 genotype

<i>BM6438</i> genotype	<i>n</i>	Milk yield (kg)		Fat				Protein			
				yield (kg)		content (%)		yield (kg)		content (%)	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
256/256	46	171	1719	294	67	4.15	0.50	223	53	3.12	0.18
256/258	17	6628	1325	288	55	4.39	0.47	211	42	3.25	0.37
256/268	55	7002	1555	294	60	4.24	0.49	221	50	3.15	0.22
258/268	20	6481	1388	289	59	4.46	0.52	201 <sup>a</sup>	43	3.18	0.19
268/268	16	7739	1579	329	53	4.32	0.51	247 <sup>a</sup>	49	3.19	0.19

<sup>a</sup>identical indexes (small letter a) designate significant differences between genotype groups at  $P \leq 0.05$

Table 3. The association between *BM6438* polymorphism and milk performance traits in the half-sib progenies of sire II with 256/268 genotype

<i>BM6438</i> genotype	<i>n</i>	Milk yield (kg)		Fat				Protein			
				yield (kg)		content (%)		yield (kg)		content (%)	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
256/256	48	6289 <sup>a</sup>	1023	289	52	4.58	0.49	216 <sup>a</sup>	32	3.45	0.21
256/258	15	6368	797	283	45	4.47	0.56	214	22	3.37	0.17
256/268	65	6393	1222	291	57	4.58	0.52	217 <sup>b</sup>	38	3.40	0.17
258/268	13	6335	1355	296	81	4.63	0.49	214	50	3.37	0.15
268/268	22	7107 <sup>a</sup>	847	311	38	4.40	0.38	244 <sup>ab</sup>	33	3.45	0.27

<sup>a</sup>identical indexes (small letter a) designate significant differences between genotype groups at  $P \leq 0.05$

Table 4. The association between *BM6438* polymorphism and milk performance traits in the half-sib progenies of sire III with 258/268 genotype

<i>BM6438</i> genotype	<i>n</i>	Milk yield (kg)		Fat				Protein			
				yield (kg)		content (%)		yield (kg)		content (%)	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
256/258	57	6462	1001	287	47	4.47	0.56	216	34	3.36	0.36
258/258	11	6539	1058	300	31	4.65	0.48	221	28	3.40	0.24
256/268	48	6310	851	287	44	4.55	0.44	215	30	3.42	0.22
258/268	28	6350	845	290	51	4.57	0.60	216	28	3.41	0.15
268/268	23	6387	1043	291	51	4.57	0.38	216	36	3.38	0.21

In the group of the progeny of sire III with 258/268 genotype the differences between genotype groups were small and statistically non-significant (Table 4).

The collective analysis of the progeny of two 256/268 sires (the progeny of sires: I and II) demonstrated a stronger phenotypic effect than that recorded separately in each family (Table 5).

Table 5. The association between *BM6438* polymorphism and milk performance traits in the half-sib progenies of sires I and II with 256/268 genotypes

<i>BM6438</i> genotype	<i>n</i>	Milk yield		Fat				Protein			
		(kg)		yield (kg)		content (%)		yield (kg)		content (%)	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
256/256	94	6721	1468	291	60	4.37	0.54	219 <sup>A</sup>	43	3.29	0.26
256/258	32	6506 <sup>a</sup>	1100	286	50	4.42	0.51	213 <sup>B</sup>	33	3.31	0.30
256/268	120	6672 <sup>b</sup>	1412	292	58	4.43	0.53	219 <sup>C</sup>	44	3.29	0.23
258/268	33	6385 <sup>c</sup>	1350	292	69	4.55	0.50	204 <sup>D</sup>	45	3.24	0.20
268/268	38	7406 <sup>abc</sup>	1210	319	45	4.35	0.44	246 <sup>ABCD</sup>	49	3.35	0.26

<sup>ABCD</sup>: identical indexes (capital letters: A, B, C, and D) designate significant differences between genotype groups at  $P \leq 0.01$ ,

<sup>abc</sup>: identical indexes (small letters: a, b, and c) designate significant differences between genotype groups at  $P \leq 0.05$

268/268 homozygotes were characterized by a highly significantly higher ( $P \leq 0.01$ ) protein yield in comparison with the remaining genotype groups. Statistically significant differences were also determined in milk yield ( $P \leq 0.05$ ). The highest milk yield was observed in cows with 268/268 genotype, while significantly lower values were recorded in genotype groups 256/258, 256/268, and 258/268. No relationship was found between *BM6438* polymorphism and the values of the remaining milk performance traits.

## DISCUSSION

Milk performance QTLs have been localized in several regions within the BTA1 structure, but to date no candidate genes responsible for milk yield and composition have been identified. This study attempted to determine the relationship between the putative QTL-linked microsatellite marker *BM6438* localized at the position 1.78 cM (AnimalQTLdb: 7127) and milk performance traits. Our attention was focused on this position because this QTL region seems to link to the candidate loci for milk performance traits. The analysis of 1cM apart from the microsatellite *BM6438* performed by MapViewer ([http://www.ncbi.nlm.nih.gov/mapview/map\\_search.cgi?taxid=9913](http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9913)) revealed neighbouring candidate loci potentially involved in the expression of analyzed traits. The ATP synthase (*ATP50*), intersectin 1 (*ITSN1*), downstream neighbour of SON (*DONSON*), SON DNA binding protein (*SON*), glycineamide ribonucleotide formyl-transferase (*GART*), transmembrane protein 50B

(*TMEM50B*), GC-rich sequence DNA-binding factor homologue (*GCFC*), synaptojanin 1 (*SYNJ1*), melanocortin 2 receptor accessory protein (*MRAP*) gene loci were located close to the *BM6438* locus.

The obtained results demonstrate that *BM6438* polymorphism differentiated the yield of milk and protein, which was highest in 268/268 homozygotes. These results are consistent with the findings of other researchers who postulated the location of QTLs responsible for milk performance traits, mainly milk yield (Nadesalingam et al., 2001; Bagnato et al., 2008) and protein yield (Georges et al., 1995; Zhang et al., 1998), in the proximal section of chromosome BTA1. The cited authors suggested that the region is flanked by microsatellite markers *AGLA17* (0.0 cM) and *CA095* (23.9 cM) and that it is also the site of dinucleotide polymorphism of *BM6438*. A study of the Polish population of Black-and-White cattle, carried out in the course of several years with the aim of improving milk performance traits through crossbreeding with Holstein-Friesian cattle, showed a trend of changes in the frequency of *BM6438* alleles with a preference to allele 268. A preliminary analysis of the relationship between *BM6438* polymorphism and the values of milk performance traits, covering a relatively large group of commercial cattle – cows of the same age in their first lactation, showed a higher fat yield and milk fat content in 268/268 homozygotes than in cows with genotypes 256/258 and 258/258 (Czarnik et al., 2005). This relationship was not confirmed in any of the half-sib groups investigated in this study. The trend of a higher protein yield in 268/268 homozygotes than



in cows of the same age with genotypes 256/258 and 258/268, reported in a previous study of Czarnik et al. (2005), was validated both in individual families (statistically significant differences) and among the entire progeny of both heterozygous sires 256/268 (highly significant differences). The advantage of 268/268 homozygotes over the remaining genotype groups in terms of much higher protein yield, associated with higher milk yield, could suggest that allele 268 is coupled with the region responsible for the studied performance traits in 256/268 heterozygous sires (I and II). This relationship was not confirmed in the progeny of the 258/268 sire (III), which could point to a different coupling phase between *BM6438* alleles and QTLs in sires.

The suitability of *BM6438* polymorphism as a QTL linked marker requires further research. The relationship between *BM6438* polymorphism and a possible causative mutation needs to be examined with special emphasis on the characteristic features of coupling in the *cis/trans* phase that determined the varied phenotypic effect of milk performance traits in cattle.

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