

Study of an association between SNP 775C>T within the bovine *ITBG2* gene and milk performance traits in Black and White cows

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ABSTRACT: The exclusive pre-selective effect of BLAD carriers reproducing in the exposed Holstein-Friesian cattle population prompted to search for the candidate gene variants of high quality of milk performance traits within the bovine *ITBG2* gene or loci linked with bovine *ITBG2* gene. Theoretical considerations indicated that the “silent mutation” C→T localized at the position of 775 bp of the gene encoding the CD18 subunit might be a potential QTL marker of high milk productivity. An association study between the polymorphism of SNP 775C>T, and the diversification of milk performance traits was carried out on the progeny of four bulls with genotypes *BL/TL* and *775C/T* and one bull with genotypes *TL/TL* and *775C/T*. The results documented statistically significant differences in the protein content percentage of milk in two half-sib families of bulls with *BL/TL* and *775C/T* genotype and one half-sib family of bull with *TL/TL* and *775C/T* genotype. It was further concluded that the polymorphism of SNP 775C>T was found to be a more efficient QTL marker than that of D128G, since in all the analysed milk performance traits for half-sib families higher values of the F coefficient were obtained for the SNP 775C>T mutation in comparison with D128G.

Keywords: BLAD carriers; point mutation; lethal genes; milk performance traits; QTL marker

Integrins constitute a family of glycoproteins responsible for cell adhesion to the extracellular matrix, vascular endothelium, and to the surface of other cells, including leukocytes and thrombocytes. They participate in the processes of leukocyte adhesion and transition through the vascular wall (Doerchuk et al., 1989; Hynes et al., 1992). Integrins are heterodimers consisting of two non-covalently bound subunits: (a) with a molecular weight ranging from 120 kDa to 180 kDa, and (b) with a molecular weight from 90 to 110 kDa. Due to the presence of b chains, adhesins have been divided into two groups, namely: b1 (*ITBG1*) and b2 (*ITBG2*) integrins. The *ITBG1* fulfil an important function in the interactions of leukocytes with proteins of the extracellular matrix and are referred to as very late antigens (VLA) that possess a common b chain denoted as a

CD29 subunit. The *ITBG2* referred to as LEUCAM receptors are localized on the cellular membrane of neutrophils and play a key role in the adhesion processes (Cox, 1997). They are composed of a common b chain referred to as a CD18 subunit and one of the four a chains (Fett et al., 2004; Zecchinon et al., 2004), namely: aL (CD11a), aM (CD11b), aX (CD11c), and aD (CD11d) (Kijas et al., 1999). Mutations of the *ITBG2* gene encoding CD18 subunit, as the main constituent of a heterodimer as an integrin particle, may induce different phenotypic effects. A functional defect of the leukocytic system in cattle referred to as BLAD (Bovine Leukocyte Adhesion Deficiency) syndrome is manifested by the elimination of animals with a recessive homozygote (*BL/BL*) genotype before they achieve sexual maturity (Shuster et al., 1992b).

It may be assumed that a significant factor that affects the worldwide penetration of the D128G mutation is the occurrence of favourable association between BLAD carrier state and milk performance traits (Czarnik, 2000). In analysing the productive value of the progeny originating from bulls as the carriers of BLAD and those not affected by the D128G mutation, Taralik (1998) observed a positive impact of *BL* allele on the contents of fat and protein in the period of two subsequent lactations. Investigations carried out in Hungary by Janosa et al. (1999) addressed the effect of heterozygous genotype *BL/TL* on the productive value of milk cows. It was observed that daughters of the bulls not burdened with the BLAD-inducing mutation had a high yield of milk and fat compared to the cows originating from BLAD carriers. Opposite tendencies were however reported for protein content, as the cows that were the progeny of BLAD carriers demonstrated a higher protein content of milk. The results obtained by Lubieniecki et al. (1999) on a selected group of half-sib progenies demonstrated a positive effect of BLAD carrier state on milk yield and fat yield in milk. A detailed analysis of the impact of D128G mutation on the diversity of milk performance traits in eight half-sib sire families with the BLAD carrier state was carried out with the positive effect of *BL* allele on milk protein content in three half-sib families (Czarnik, 2000). The obtained results indicate that the positive effect of BLAD carrier may be restricted to some individuals. Thus, it is likely that not only the D128G mutation but some other genetic factor, linked with D128G, determines the selective prevalence of BLAD carriers, which prompted a search for markers of high value milk performance traits in the space of *CD18* gene or loci linked with *CD18*. Theoretical considerations indicated that the “silent mutation” C→T localized at the 775 bp position of the *ITGB2* gene (SNP 775C>T) might be a potential marker of high milk yield (Shuster et al., 1992a). The detection of QTL for milk performance traits is still a contentious issue. Investigations carried out to date have demonstrated the presence of QTL markers on all bovine autosomes (Georges et al., 1995; Taylor et al., 1998; Mosig et al., 2001). Based on current knowledge, it may be stated that no candidate genes, responsible for the manifestation of milk performance traits, have been found in *BTA1* so far. In contrast, three QTL regions on *BTA1* for the milk performance traits were reported (Nadesalingam et al., 2001). The aim of this study

was to validate a hypothesis assuming the possibility of an association between the polymorphism of SNP 775C>T and the diversification of milk performance traits in cattle.

MATERIAL AND METHODS

Animals

Experimental materials comprised the first lactation records from 172 half-sib progenies originating from four Black-and-White bulls with *BL/TL* and *775C/T* genotypes, and 106 half-sib progenies originating from one bull with *TL/TL* and *775C/T* genotypes. All investigated animals belonged to one large herd with uniform environmental and feeding conditions.

Laboratory procedure

DNA isolation: DNA of the analysed population was extracted from the blood and semen using MasterPure™ (Epicentre, USA) and Wizard® (Promega, USA) genomic DNA purification kit. The PCR reactions were carried out in a 25 µl volume containing 50 pmol of each primer set, 10× optimized buffer containing MgCl₂, 2mM each of dNTP, enhancer and 0.2 U of *Tfl* DNA polymerase (Epicentre, USA) and 50 ng of genomic DNA. Touch down DNA amplification protocols (Don et al., 1991) were applied to obtain the respective PCR products to identify the carriers of D128G mutation and SNP 775C>T. The PCR programs started with initial denaturation at 94°C for 4 min followed by 1 min denaturation at 94°C, 1 min annealing at started 70°C to a touch down annealing temperature of 60°C and 1 min extension at 72°C, which ended by 30 cycles and a final elongation step at 72°C, for 10 min, using Rapidcyclor Idaho Technology, USA.

The primer sequences to amplify the 367 bp fragment (D128G mutation) and 108 bp fragment (SNP 775C>T) of bovine *ITGB2* gene were as follows.

D128G_F: 5'-AGGTCAGGCAGTTGCCTTCAA-3'

D128G_R: 5'-GGGGAGCACCGTCTTGTCCAC-3'

SNP 775C>T_F: 5'-GAGGAAATCGGCTGGCGCAATG-3'

SNP 775C>T_R: 5'-GTCATT GGGGGTGAGGATG-3'

Table 1. Relationship between the polymorphism of SNP 775C>T in bovine *ITBG2* gene and milk performance traits ($\bar{x} \pm \text{SD}$) of the first lactation in half-sib sire family 1

	Genotypes D128G		Genotypes SNP 775C>T		
	<i>BL/TL</i> <i>n</i> = 14	<i>TL/TL</i> <i>n</i> = 30	<i>775T/T</i> <i>n</i> = 4	<i>775C/T</i> <i>n</i> = 17	<i>775C/C</i> <i>n</i> = 23
Milk yield (kg)	4741 ± 837	4845 ± 944	4514 ± 841	4874 ± 967	4818 ± 893
<i>F</i>	0.125			0.249	
Fat yield (kg)	218 ± 40	217 ± 46	215 ± 44	221 ± 42	215 ± 47
<i>F</i>	0.003			0.111	
Protein yield (kg)	167 ± 26	165 ± 34	165 ± 7	169 ± 35	164 ± 33
<i>F</i>	0.027			0.108	
Fat content (%)	4.60 ± 0.28	4.48 ± 0.41	4.76 ± 0.21	4.54 ± 0.31	4.46 ± 0.42
<i>F</i>	1.012			1.608	
Protein content (%)	3.46 ± 0.20	3.42 ± 0.24	3.66 ± 0.29	3.54 ± 0.26	3.40 ± 0.16
<i>F</i>	0.311			3.967 ^x	

The values marked with ^xletter showed a highly significant effect ($P \leq 0.05$)

The obtained PCR products were digested with *TaqI* (Fermentas, Lithuania) and *Fnu4 HI* restrictionases (BioLabs, UK) and for the identification of genotypes at D128G mutation and SNP 775C>T the digests were separated on 3% agarose gel (Cortified™ Low Range Ultra Agarose, Bio-Rad) with ethidium bromide, and then visualised by a UVP trans-illuminator 7500 system (UK).

The results of milk performance evaluation were compiled taking into account differences in milk yield as well as fat and protein contents of milk in cows kept in different herds. An association study between SNP 775C>T and the diversity of corrected values of milk performance traits was computed using the ANOVA procedure (Statistica 6, Statsoft Inc).

Statistics

The milk performance traits were analysed on the basis of 305 days of the first lactation records.

RESULTS AND DISCUSSION

The results shown in Tables 1, 2, 3, 4 and 5 represent an attempt to prove a hypothesis assuming the

Table 2. Relationship between the polymorphism of SNP 775C>T in the gene encoding bovine *ITBG2* and milk performance traits ($\bar{x} \pm \text{SD}$) of the first lactation in half-sib sire family 2

	Genotypes D128G		Genotypes SNP 775C>T		
	<i>BL/TL</i> <i>n</i> = 21	<i>TL/TL</i> <i>n</i> = 25	<i>775T/T</i> <i>n</i> = 5	<i>775C/T</i> <i>n</i> = 27	<i>775C/C</i> <i>n</i> = 14
Milk yield (kg)	5014 ± 974	4949 ± 738	4329 ± 777	5072 ± 858	5031 ± 792
<i>F</i>	0.067			1.725	
Fat yield (kg)	220 ± 46	214 ± 30	189 ± 29	223 ± 43	216 ± 28
<i>F</i>	0.286			1.678	
Protein yield (kg)	168.1 ± 36.9	163 ± 22	139 ± 17	171 ± 32	164 ± 22
<i>F</i>	0.338			2.761	
Fat content (%)	4.39 ± 0.45	4.42 ± 0.36	4.38 ± 0.60	4.39 ± 0.40	4.29 ± 0.31
<i>F</i>	0.082			0.245	
Protein content (%)	3.35 ± 0.27	3.29 ± 0.21	3.22 ± 0.28	3.37 ± 0.23	3.25 ± 0.21
<i>F</i>	0.553			1.556	

Table 3. Relationship between the polymorphism of SNP 775C>T in the gene encoding bovine *ITBG2* and milk performance traits ($\bar{x} \pm \text{SD}$) of the first lactation in half-sib sire family 3

	Genotypes D128G		Genotypes SNP 775C>T		
	<i>BL/TL</i> <i>n</i> = 20	<i>TL/TL</i> <i>n</i> = 22	<i>775T/T</i> <i>n</i> = 4	<i>775C/T</i> <i>n</i> = 26	<i>775C/C</i> <i>n</i> = 12
Milk yield (kg)	5244 ± 1154	5250 ± 1376	4722 ± 1327	5342 ± 1268	5217 ± 1285
<i>F</i>	0.000			0.412	
Fat yield (kg)	220 ± 59	223 ± 61	205 ± 56	225 ± 64	221 ± 54
<i>F</i>	0.025			0.202	
Protein yield (kg)	168 ± 39	166 ± 45	153 ± 43	169 ± 43	168 ± 41
<i>F</i>	0.008			0.249	
Fat content (%)	4.20 ± 0.45	4.25 ± 0.40	4.34 ± 0.44	4.22 ± 0.44	4.23 ± 0.40
<i>F</i>	0.117			0.361	
Protein content (%)	3.20 ± 0.20	3.17 ± 0.22	3.23 ± 0.12	3.16 ± 0.20	3.23 ± 0.24
<i>F</i>	0.200			0.511	

possibility of an association between SNP 775C>T and the milk performance traits in cattle. The results cover the progeny of four bulls with D128G *BL/TL* and *775C/T* genotypes and one bull with D128G *TL/TL* and *775C/T* genotypes. An association study was carried out alternatively, considering the polymorphism of D128G and SNP 775C>T in a coordinate manner.

The results of the present study demonstrated a statistically significant association between SNP 775C>T and protein content of milk in three half-sib families (1, 4 and 5). In these analysed half-sib progeny groups, genotype *775T/T* had the highest milk protein content value followed by

genotypes *775T/C* and *775C/C* (Tables 1, 4 and 5). In contrast, no statistically significant differences were reported in two half-sib families (2 and 3) for the analysed milk performance traits that would be determined by either D128G genotype or SNP 775C>T (Tables 2 and 3). An interesting part of the results is the conformity of observations indicating the occurrence of an association between SNP 775C>T within the bovine *ITBG2* gene and the diversified content of protein, with a simultaneous lack of associations with other milk performance traits. Comparing the results of ANOVA, it may be speculated that in comparison with the D128G mutation, SNP 775C>T is a more reliable marker

Table 4. Relationship between the polymorphism of SNP 775C>T in the gene encoding bovine *ITBG2* and milk performance traits ($\bar{x} \pm \text{SD}$) of the first lactation in half-sib sire family 4

	Genotypes D128G		Genotypes SNP 775C>T		
	<i>BL/TL</i> <i>n</i> = 17	<i>TL/TL</i> <i>n</i> = 22	<i>775T/T</i> <i>n</i> = 6	<i>775C/T</i> <i>n</i> = 17	<i>775C/C</i> <i>n</i> = 16
Milk yield (kg)	5044 ± 961	5055 ± 983	4823 ± 964	5114 ± 1001	5031 ± 935
<i>F</i>	0.006			0.717	
Fat yield (kg)	218 ± 47	218.6 ± 43	213 ± 40	221 ± 49	217 ± 40
<i>F</i>	0.003			0.367	
Protein yield (kg)	171 ± 32	174.0 ± 26	171 ± 29	171 ± 24.0	166 ± 33
<i>F</i>	0.130			0.199	
Fat content (%)	4.32 ± 0.39	4.32 ± 0.41	4.41 ± 0.29	4.31 ± 0.40	4.30 ± 0.47
<i>F</i>	0.000			0.264	
Protein content (%)	3.38 ± 0.18	3.32 ± 0.17	3.58 ± 0.20	3.33 ± 0.19	3.28 ± 0.16
<i>F</i>	0.267			3.862 ^x	

The values marked with ^xletter showed a highly significant effect ($P \leq 0.05$)

Table 5. Relationship between the polymorphism of SNP 775C>T in the gene encoding bovine *ITBG2* and milk performance traits ($\bar{x} \pm \text{SD}$) of the first lactation in half-sib sire family 5

	Genotypes SNP 775C>T			Total <i>n</i> = 106
	775T/T <i>n</i> = 6	775C/T <i>n</i> = 60	775C/C <i>n</i> = 40	
Milk yield (kg)	6393 \pm 1439	6578 \pm 1231	6558 \pm 1140	6560 \pm 1198
<i>F</i>		0.060		
Fat yield (kg)	278 \pm 56	299 \pm 52	295 \pm 59	296 \pm 55
<i>F</i>		0.470		
Protein yield (kg)	217 \pm 45	212 \pm 38	219 \pm 37.0	215 \pm 37
<i>F</i>		0.249		
Fat content (%)	4.35 \pm 0.22	4.55 \pm 0.41	4.50 \pm 0.50	4.52 \pm 0.44
<i>F</i>		0.620		
Protein content (%)	3.39 \pm 0.18	3.23 \pm 0.19	3.34 \pm 0.18	3.29 \pm 0.19
<i>F</i>		4.036 ^x		

The values marked with ^xletter showed a highly significant effect ($P \leq 0.05$)

of QTLs for milk protein content, as for all the analysed traits and all progeny groups the obtained *F* values were higher for SNP 775C>T than for D128G. In addition, one half-sib family with a bull genotyped as homozygous *TL/TL* demonstrated the identical tendency for the diversified protein content of milk as the half-sib families originating from heterozygous bulls with *BL/TL* genotypes.

Investigations into the polymorphism within the bovine *ITBG2* gene were dominated by the need for the identification of BLAD carriers. Most papers have eluded the possibility of applying the D128G mutation as a QTL marker. Scant attempts to verify D128G as the QTL marker of milk performance traits were carried out by Taralik (1998), Janosa et al. (1999) and Czarnik (2000); they demonstrated a significant association between the lethal allele *BL* and higher protein content of milk. Studies by Czarnik (2000) involving a local population of Black-and-White cattle confirmed an interaction between the D128G genotype and environmental factors. The *BL* allele effect was beneficial if the cows were kept under good conditions, whereas under poor conditions it was negative. Analyses of the progeny of bulls – *BL* carriers demonstrated that the presence of the *BL* allele induced positive phenotypic effects only in some cases. It was assumed that the search for positive QTL markers of milk performance traits should consider a possibility of the occurrence of a genetic factor other than D128G linked or functionally bound with the CD18 subunit. In the present study it was assumed that SNP 775C>T within the

bovine *ITBG2* gene could be the factor capable of differentiating the milk performance traits. So far, the polymorphism of this mutation has been explored only in a Polish population of Black-and-White cattle (Czarnik et al., 2004). No other attempts have ever been made to apply this SNP 775C>T mutation as a QTL marker. The final evaluation of the usability of the bovine *ITBG2* gene polymorphism as a marker of milk performance traits requires further study. An alternative hypothesis may be considered as well, namely, that SNP 775C>T within the bovine *ITBG2* gene is not only a causative agent of the diversified protein content of cow milk but also it is in linkage disequilibrium with a yet unknown causal mutation. Subsequent investigations should be focused on the scanning of chromosome BTA1 and an analysis of the effect of mutations, localized within encoding genes, functionally linked with lactation and synthesis of milk components.

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Received: 2006–01–03

Accepted after correction: 2006–11–01

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