

Short Communication: Effect of Leptin Gene Polymorphisms on Breeding Value for Milk Production Traits

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ABSTRACT

New molecular techniques focused on genome analysis open new possibilities for complex evaluation of economically important traits in farm animals. Milk production traits are typical quantitative characteristics controlled by a number of genes. Mutations in their sequences may alter animal performance as well as their breeding values. In this study, we investigated the effect of 3 restriction fragment length polymorphisms (RFLP): *HphI*, *Kpn2I*, and *Sau3AI* in the leptin gene, on bull breeding values for milk, fat, and protein yield, and fat and protein content. One hundred seventeen Polish Black and White AI bulls were genotyped. Pedigree analysis indicated a relatively close relationship between the bulls. Statistical analysis indicated that the *HphI* polymorphism has a significant effect on milk and protein yield. Animals with the TT genotype had approximately 2× higher estimated breeding values for milk and protein yields. No effect was found for the other 2 polymorphisms.

(Key words: dairy cattle, leptin polymorphism, milk traits, breeding value)

Abbreviation key: BTA = *Bos taurus* autosome.

Milk production traits are quantitative traits controlled by numerous genes and environmental factors. Recent genome scans using Dairy Bull DNA Repository grandsire families found strong evidence for QTL on *Bos taurus* autosome (BTA) 3 (affecting protein and fat percentage), on BTA 6 (affecting protein and fat percentage), on BTA 14 (affecting fat percentage), and on BTA 20 (affecting protein percentage) (Ashwell et al., 2004). Lindersson et al. (1998) reported a QTL for milk production traits on chromosome 4 in a region where the leptin gene and the serum amylase-1 gene are located. The leptin gene itself is considered a poten-

tial QTL, influencing different production traits in cattle, for example, meat production (Buchanan et al., 2002), milk performance (Silva et al., 2002; Buchanan et al., 2003), and reproduction (Gonzalez et al., 2000). Several polymorphisms in this gene have been found. In exon 2, two RFLP were described: *ClaI* (an A/T substitution resulting in an AA change from tyrosine to phenylalanine) (Lagonigro et al., 2003), and *Kpn2I* (a C/T substitution resulting in an AA change from arginine to cysteine) (Buchanan et al., 2002). Another 2 RFLP were identified in exon 3: *NruI* (a C/T substitution resulting in a change from valine to alanine) (Lagonigro et al., 2003), and *HphI* (a C/T substitution resulting in a change from alanine to valine) (Haegeman et al., 2000). Moreover, a *Sau3AI* polymorphism in intron 2 was found (Pomp et al., 1997). In addition, in the BovMap database (<http://locus.jouy.inra.fr>), 19 other single nucleotide polymorphisms are listed.

The aim of this study was to confirm a suggested influence of *Kpn2I*, *HphI*, and *Sau3AI* polymorphisms on milk production. Studies were performed on 117 Polish Black and White proven bulls with an average reliability of 93.2% (ranging from 71 to 99). Estimated breeding values for milk, fat, and protein yield, and fat and protein content from official Polish evaluations were used. Animal pedigrees used in this study were complete on the sire and dam side. Over 62% of the ancestors up to the seventh generation were known. DNA was extracted from blood using the phenol-chloroform method (according to standard protocol). For each PCR reaction (conditions specified in Table 1), 0.5 U of Taq polymerase (Finnzymes, Espoo, Finland), 200 μM of dNTP (Eppendorf, Hamburg, Germany), and 0.3 μM of primer were used. The RFLP were analyzed as follows. For *Kpn2I*, a 94-bp PCR product was digested for 2.5 h at 55°C with 2 U of restriction enzyme (Fermentas, Vilnius, Lithuania), and analyzed on a 3% agarose gel. Digestion resulted in genotypes CC (75 and 19 bp), CT (94, 75, and 19 bp), and TT (94 bp, uncut product). For *Sau3AI*, an 1820-bp PCR product was digested overnight at 37°C with 3 U of endonuclease (Fermentas) and analyzed on 2% agarose gel. Digestion resulted in genotypes AA (730, 690, and 400 bp), AB (730, 690, 400,

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Table 1. PCR conditions.

Polymorphism	Primers	Initial denaturation	Denaturation	Annealing	Extension	No. of cycles	Final extension	Product size
<i>Kpn2I</i>	Buchanan et al. (2002)	94°C/2 min	94°C/45 s	59°C/45 s	72°C/1 min	35	72°C/5 min	94 bp
<i>Sau3AI</i>	Pomp et al. (1997)	94°C/5 min	94°C/1 min	57.3°C/30 s	72°C/1 min	40	72°C/10 min	1820 bp
<i>HphI</i>	Haegeman et al. (2000)	95°C/1 min	95°C/5 min	60°C/30 s	72°C/1 min	30	72°C/10 min	331 bp

310, and 90 bp), BB (730, 690, 310, and 90 bp), and a rare AC polymorphism resulting from an additional restriction site (the 690-bp fragment was digested into 470- and 220-bp fragments). For *HphI*, a 331-bp PCR product was digested overnight at 37°C with 3 U of the restriction enzyme (NewEngland BioLabs, Frankfurt, Germany), and analyzed on a 2% agarose gel. Digestion revealed 3 genotypes, CC (undigested, 331 bp), CT (331, 311, and 20 bp), and TT (311 and 20 bp).

All genotypes were distributed according to the Hardy–Weinberg law and the frequencies of the alleles were as follows. *Kpn2I*: C = 0.54, T = 0.46; *HphI*: C = 0.66, T = 0.34; and *Sau3AI*: A = 0.86, B = 0.11, C = 0.03. The effect of genotype on the breeding values of bulls for 5 traits was tested by analyzing least square means within the one-way ANOVA. The MULTTEST procedure of SAS (SAS, 1999) was used to allow for *P*-value adjustment by permutation.

The results showed that the *HphI* polymorphism (the TT genotype) had an effect on the breeding values of yield traits (Table 2). Animals with the TT genotype had approximately 2× higher EBV for milk and protein yields (for both traits *P* = 0.006). Fat yield for this genotype also tended to be higher (*P* < 0.1). The *HphI* restriction site resulting in a change from alanine to valine is located at the conserved region of the β helix of the leptin protein. Because alanine and valine have similar nonpolar aliphatic R-groups, the substitution should not affect the protein structure or binding to its receptor. This polymorphism should not, therefore, directly influence production traits, but may be linked with other unknown milk production QTL in the vicinity. No effect on fat and protein content suggests that differences in fat and protein yields are generally re-

lated to increased milk yield. We did not find associations between the *Kpn2I* and *Sau3AI* polymorphisms and production traits, in contrast with the results published by Liefers et al. (2002). Liefers et al. (2002) did not find any effect of the *HphI* polymorphism, although they pointed to *Sau3AI* as a possible marker for milk and protein yield. Similarly, Buchanan et al. (2003) demonstrated a strong influence of the *Kpn2I* polymorphism (the TT genotype) on milk and protein yield, which was not confirmed in this study. The presence of a rare C allele (*Sau3AI*) (associated with some cattle breeds, e.g., Gelbvieh, Simmental, and Angus; Pomp et al., 1997) was also related to fat and protein content in the Polish Black and White cattle (Zwierzchowski et al., 2002). Our studies do not support these relationships. Such discrepant results may be due to a number of factors, including the relatively small number of animals studied (117 bulls), population differences or breed composition, the small number of animals with the TT genotype (12), and the simple design of our experiment. On the other hand, the average reliability of bull proofs indicates a rather high number of daughters used in the genetic evaluation. In addition to genotype, many other factors influence milk yield and composition. These include environmental factors, the cow's age, lactation parity and stage, and the animal's health with special reference to the mammary gland (Mackle et al., 1998). Although many nongenetic factors are included in the genetic evaluation model, they may still interfere with the genetic component.

Pedigree analysis revealed that a group of 12 bulls genotyped as TT for *HphI* had 2 common ancestors, of which one appeared in the third or fourth generation. The average relationship among these bulls was 6.75%,

Table 2. Mean EBV (standard deviation) for production traits of bulls with different *HphI* genotypes. Values denote deviations from the official genetic base defined as the mean breeding values of cows born in 1995.

Genotype	No. of bulls	Milk yield (kg)	Fat yield (kg)	Protein yield (kg)	Fat content (% × 100)	Protein content (% × 100)
CC	50	447.06 (465.08) ^A	19.37 (16.01) ^a	14.43 (14.27) ^A	1.67 (25.04)	−0.02 (12.12)
CT	55	511.77 (454.88) ^A	21.51 (14.98) ^a	14.48 (13.14) ^A	0.96 (25.63)	−3.97 (9.59)
TT	12	913.07 (332.15) ^B	31.33 (16.23) ^b	28.17 (9.62) ^B	−8.88 (22.19)	−0.62 (12.20)
Probability		0.006	0.065	0.006	0.496	0.997

^{A,B}Means sharing a common (uppercase) superscript are not significantly different (*P* < 0.01); ^{a,b}Tendency (*P* < 0.1).

suggesting that these animals may have many genes in common. Further studies of pedigrees showed that the average relationship between all studied bulls was 4.99% and one bull (the common ancestor of the 12 TT bulls) contributed 18.4% to this average relationship. Such a close relationship makes it difficult to draw unambiguous conclusions. The contradictory results of the effect of the leptin gene polymorphisms on milk production may reflect the presence of population specific haplotypes and other unknown milk production QTL.

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