Relationships of Polymorphisms for Growth Hormone and Growth Hormone Receptor Genes with Milk Production Traits for Italian Holstein-Friesian Bulls

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ABSTRACT

Allelic variation in the structural or regulatory sequences of growth hormone and its receptor genes might directly or indirectly affect milk traits. This possibility prompted us to investigate the eventual relationships of restriction fragment length polymorphisms at the locus of bovine growth hormone (using *Tag*I and *Msp*I restriction enzymes) and its receptor (using *Taq*I restriction enzyme) to PTA of milk production traits of bulls. Ninety-one Italian Holstein-Friesian bulls were used in this experiment, and data were analyzed with a fixed linear model. The restriction fragment length polymorphisms at the growth hormone locus did not affect the milk traits studied. Six restriction enzyme *Taq*I bands of 7.1, 6.2, 5.7, 5.4, 4.2, and 3.3 kb with nine patterns were observed after hybridization by a cDNA probe containing the coding sequences for the intracellular C-terminal part of the receptor. The effect of this polymorphism on PTA for milk protein percentage was highly significant and was favorable for the rare (6.6%) 5.7- and 5.4-kb pattern. Our results indicate that further study is needed to explain the DNA polymorphism and to obtain more definite conclusions about effects on milk traits.

(**Key words**: milk traits, growth hormone, growth hormone receptor, restriction fragment length polymorphism)

Abbreviation key: **GH** = growth hormone, **GH-*MspI*** = generated fragments using the restriction enzyme *Msp*I on the GH gene, **GHR** = GH receptor, **GH-*TaqI*** = generated fragments using the restriction enzyme *Taq*I on the GH gene. **GHR-*TaqI*** = generated fragments using the restriction enzyme *Taq*I on the GHR gene, **MAS** = marker-assisted selection, **QTL** = quantitative trait loci, **RFLP** = restriction fragment length polymorphism.

INTRODUCTION

The expanding technologies of molecular biology permit the investigation of variation in primary gene structure and cause animal breeders to approach selection decisions in a new and challenging way. Current developments in molecular genetics permit identification of single loci and modifications of their sequences. Substitutions, insertions, or deletions at restriction enzyme cleavage sites may produce changes in the resulting length of DNA fragments known as restriction fragment length polymorphisms (**RFLP**) (8). The RFLP may directly affect gene expression by changing the splicing of mRNA, stability of mRNA, rate of gene transcription, or the sequence of the gene product. The RFLP may also serve as genetic markers if linked to quantitative trait loci (**QTL**) (7, 33). Hence, the determination of the contribution (direct or as a marker) of RFLP to production traits before their subsequent use in selection schemes is of great interest.

Various studies have shown that a number of single genes that are inherited in a simple Mendelian manner and that can be screened for and identified in the animal are associated with quantitative traits of economic importance. Examples are associations of lactation production with milk protein alleles (9, 19), α-amylase-1, blood B antigen system, major histocompatibility complex, and genetic defects (12).

Recently, the contribution of genetic markers to genetic improvement of dairy cattle has been studied

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by Kashi et al. (22) and Meuwissen and Van Arendonk (27). Kashi et al. (22) studied progeny-testing schemes that used genetic markers to preselect young bulls entering progeny test and to predict an increase in genetic gain of 15 to 30%. Between 5 and 20 QTL coding for milk production were traced from grandparents to the young bulls. With a different approach, Meuwissen and Van Arendonk (27) studied the effects of inclusion of marker information for determination of PTA for bulls in both progeny-testing and nucleus breeding schemes using multiple ovulation and embryo transfer. Additional returns resulted from increased accuracy of prediction of PTA. Prediction of genetic gain with genetic markers needs further investigation because some aspects were not considered, such as reduction of generation intervals and the overlap between response from marker-assisted selection (**MAS**) and classic selection. Furthermore, MAS would be more accurate if all information, not just information on grandparents, is included.

Growth hormone (**GH**) has a key role in mammary gland development and milk production (1, 5, 11). Also, GH has a tissue-specific action that is either direct or indirect via IGF-I, and the effect of this action depends on the GH receptor (**GHR**) and several other hormones (4). Some parameters of GH secretion, for example, frequency of GH peaks and total daily secretion of GH, were associated with higher PTA in studies comparing GH secretion of more highly selected and control lines of cattle (15, 28), sheep (13), and pigs (3). Klindt (23) found that the inclusion of parameters for GH secretion in the prediction of PTA for dairy bulls improved the accuracy of predictors of progeny performance. In studies of British Friesian calves, Løvendahl et al. (25) found a positive association between GH release induced by GH-releasing factor or thyrotropin-releasing hormone and PTA for milk production.

Allelic variation in the structural or regulatory sequences of the GH or the GHR genes would be of interest because of possible direct or indirect effects on milk production and growth performance. Also, variations in introns or flanking sequences have potential usefulness as genetic markers. The RFLP have been detected at the GH gene in cattle, and a restriction map of this gene and its flanking sequences has been developed (10, 16). Two RFLP have been identified around the bovine GH gene: 1) an insertion-deletion of 0.9 to 1.0 kb in the 3′ region and 2) an *Msp*I polymorphic site in the third intron (6, 10, 16, 19, 36).

Consequently, because GH is involved in lactation, GH and GHR genes have potential as markers for genetic variation in milk production traits; linkages of RFLP around these genes with QTL for milk traits may be possible.

The objective of this study was to investigate the effects of RFLP for GH and GHR on the PTA for milk production traits of Italian Holstein-Friesian bulls and genetic indexes grouping PTA for several traits.

MATERIALS AND METHODS

Bulls

Genomic DNA was extracted from semen of 91 registered Italian Holstein-Friesian bulls that were commercially available as described by Lucy et al. (26). The PTA from July 1994 evaluation were obtained from the milk registration codes of the Italian Holstein-Friesian breeder association ANAFI (Associazione Nazionale Allevatori Frisona Italiana, Cremona, Italy). The PTA for linear conformation traits and final scores were also obtained and were half of the EBV.

The PTA for production traits were obtained with a single-trait animal model using repeated records (more than 7,400,000 records available in 1995) as described by Aleandri et al. (2) and Canavesi (1995, personal communication). The model was

$$y = HYSP_i + pe_j + a + e$$

where HYSP = group i for herd-year, season, and parity (parity is 1 or >1); pe = permanent environmental effect of the cow j; a = additive polygenic genetic effect (2 × PTA), and e = residual error for each observation.

Different preadjustments included lactation extended to 305 d for twice daily milkings, mature equivalent adjusted within parity and area of production (to 84 mo of age, calving during January), days open (to 120 d), and phenotypic adjustment for heterogenous variances. Heritability and repeatability were assumed to be 0.25 and 0.5, respectively, for all traits.

Also analyzed were the PTA for final conformation scores and the genetic indexes: ILQM and ICM. The ICM is obtained by combining additive PTA for scores of linear conformation traits expressed as standardized PTA with mean of 0 and standard deviation of 1. The ILQM is the selection decision criterion for the Italian Holstein-Friesian cattle population; its formula is as follows:

$$ILQM = (0.90 \times ILQ) + (180 \times ICM),$$

where ILQ = 4.5 [–0.173 (2 × PTA for milk) + (2 × PTA for fat) + 11.3 (2 × PTA for protein)], and ICM = [0.18 (standardized PTA for udder attachment) +

0.16 (standardized PTA for udder height) + 0.05 (standardized PTA for rear udder width) + 0.20 (standardized PTA for ligament) + 0.25 (standardized PTA for udder depth) + 0.16 (standardized PTA for teat size)].

Because all of these PTA were obtained with an animal model, relationships between animals are included. Our strategy was to test the polygenic additive genetic merits for their association with certain patterns. Means and standard deviations of PTA for milk production traits of the bulls sample are presented in Table 1.

Southern Blot Protocol

The RFLP at the GH gene using *Taq*I or *Msp*I restriction enzymes (**GH-*Taq*I** and **GH-*Msp*I**, respectively) and RFLP at the GHR gene using the *Taq*I restriction enzyme (**GHR-*Taq*I**) were revealed by Southern blot analysis as described by Sneyers et al. (35).

The GH probe was produced in our laboratory as follows: a 1505-bp *Bam*HI-*Sma*I fragment, which covers the bovine GH gene from base 7 of exon 1 to base 21 of the last exon 5 described by Gordon et al. (14), was amplified by polymerase chain reaction from bovine genomic DNA and cloned in plasmid pBluescript KS+. Methods of ligation, transformation of *Escherichia coli* (line JM 105), plasmid amplification, and plasmid purification followed the method of Sambrook et al. (30). The insert was excised from the vector by digestion with *Bam*HI and *Sma*I restriction enzymes, separated from plasmid DNA by agarose gel electrophoresis, purified by use of a commercial kit (Gene Clean, Westburg, The Netherlands), and utilized as a probe after verification of the nucleotide sequence. The GHR probe was produced as performed for the GH probe and consisted of a 1040-bp *Eco*RI-*Eco*RI insert cDNA (34), which contained the coding sequences (from position +1194 to +2233) for the C-terminal intracellular region of the receptor (17).

Statistical Analysis

Statistical analysis was performed using the GLM procedure of SAS (31). The following linear model was used to look for associations between GH-*Taq*I, GH-*Msp*I, or GHR-*Taq*I polymorphisms and PTA for milk traits:

$$y_{ij} = \mu + g_j + e_{ij}$$

where y_{ij} = PTA or genetic index of bull i; μ = mean of all observations; g_j = fixed effect associated with GH-*Taq*I, GH-*Msp*I, or GHR-*Taq*I pattern j; and e_{ij} = random residual effect.

Dependent variables were PTA for milk, fat, and protein production and percentages of fat and protein. Additional analysis were performed on PTA for final conformation scores and genetic indexes ICM and ILQM.

RESULTS AND DISCUSSION

RFLP

Two restriction fragment bands of 6.2 and 5.2 kb, denoted by A and B, were detected using the *Taq*I restriction enzyme and a GH probe (Figure 1). Two GH-*Taq*I patterns, AA and AB, were observed with frequencies of 70.3 and 29.7%, respectively. The GH-*Taq*I bands seemed to be identical to those previously reported in other studies and explained by an insertion-deletion of 0.9 to 1 kb (10, 16, 29) for Holsteins. Also, in our study, the frequencies of the

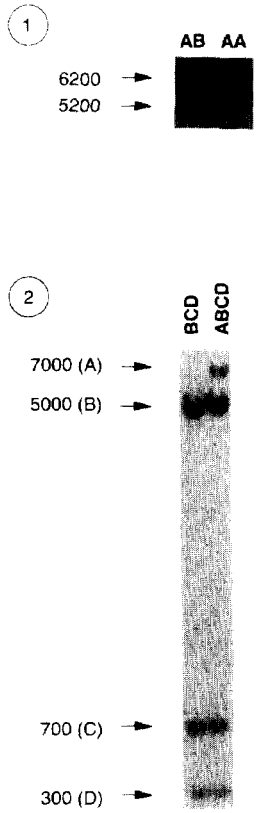

Figure 1. The patterns generated using the restriction enzymes 1) *Taq*I and 2) *Msp*I on the growth hormone gene observed for 91 Holstein-Friesian Bulls. The sizes of digested fragments are on the left, and the patterns are at the top. Fragment length (in base) was estimated relative to the DNA size markers λ/*Hin*dIII and φX174 DNA/*Hae*III fragments.

TABLE 1. Means of PTA for milk production and conformation traits of 91 Italian Holstein-Friesian bulls.

Trait	$\bar{X}$	SD	Minimum	Maximum
Milk, kg	+811	190	+324	+1261
Fat, kg	+28.24	7.17	+6.00	+96.50
Protein, kg	+27.02	5.89	+11.00	+44.50
Fat, %	−0.0103	0.0798	−0.1650	+0.2200
Protein, %	+0.0319	0.0354	−0.0750	+0.1200
FCS[1]	0.4220	0.3159	−0.2550	1.1650
Accuracy[2]	0.86		0.76	0.99
ICM[3]	0.6560	1.0519	−1.9200	3.3400
ILQM	+1683	+360	+741	+2517
Rank[4]	80	13	45	99

[1]Final conformation score.

[2]Mean accuracy of PTA (because heritabilities are different).

[3]ICM = Genetic index combining principal linear conformation traits; ILQM = genetic index combining PTA for milk, fat, and protein yields and linear conformation traits.

[4]Rank of bulls based on the ILQM index: for example, a bull with rank of 90 is classified in the best 10% of evaluated bulls.

GH-*Taq*I bands A (85.2%) and B (14.8%) were not very different from those found by Rocha et al. (29) (88 and 12%) for Holsteins.

Southern blot analysis for RFLP yielded *Msp*I restriction enzyme DNA fragments of 7.0, 5.0, 0.7, and 0.3 kb, denoted arbitrarily by A, B, C, and D, respectively. Two hybridization GH-*Msp*I patterns, ABCD and BCD, with frequencies of 34.1 and 65.9%, respectively, were revealed (Figure 1). The genetic origin of *Msp*I RFLP at the bovine GH gene was demonstrated in other studies to be two *Msp*I restriction sites in the fifth exon and the third intron, and the second site was polymorphic (10, 16, 19, 20, 36); the polymorphic *Msp*I restriction site was localized at the 5′ position +830 in the third intron (20). Using the same procedure as in our study (Southern blot analysis for RFLP), Cowan et al. (10) found, in a sample of 14 Holstein bulls, three bands of 0.45, 0.7, and 0.8 kb; two patterns were distinguished by the presence or absence of the 0.7-kb band. Our C and D bands probably correspond to the 0.7- and 0.45-kb bands reported by those researchers (10). However, Cowan et al. (10) did not observe the A and B bands, and further studies are needed to characterize the molecular event responsible for their presence, which might be an insertion-deletion nucleotide sequence. This disparity might be explained by differences in sample sizes or in the genetic background of the animals examined.

Highly polymorphic restriction fragments were observed at the locus of the bovine GHR using the *Taq*I restriction enzyme. The six bands detected were denoted A, B, C, D, E, and F for bands of 7.1, 6.2, 5.7, 5.4, 4.2, and 3.3 kb, respectively; nine patterns occurred (Figure 2). Most bulls exhibited the BC (29.7%) or the BCD (33.0%) patterns, and the frequencies of the remaining patterns varied from 2.2 to 9.9% (Table 3). We have no knowledge of any previously reported RFLP at the bovine GHR locus. Høj et al. (20) did not observe any RFLP with the restriction enzymes *Taq*I, *Bgl*II, *Dra*I, *Eco*RV, *Hin*dIII, *Pst*I, or *Pvu*II and hybridization with a rabbit GHR cDNA probe for 58 Red Danish calves and 32 Norwegian Red heifers.

Relationship of RFLP to Milk Production

The results of the statistical analysis are presented in Tables 2 and 3. Statistical models and analyses similar to those in this study were previously used for studies of RFLP effects on PTA and genetic indexes for production traits (26, 32).

The effects of either GH-*Taq*I or GH-*Msp*I patterns on PTA and indexes for milk production and type score were not significant (Table 2). To our knowledge, all previous studies of dairy cattle were restricted to comparisons of frequencies of GH-*Taq*I or GH-*Msp*I allele between different lines or breeds; those studies did not investigate the effects of the alleles on milk traits. Høj et al. (21) reported a significant difference in frequencies of insertion (I) and deletion (D) alleles, which corresponded to the GH-*Taq*I bands A and B, respectively, between lines of Red Danish and Norwegian Red selected for high and low milk fat production; the D allele was more frequent in the lines selected for high milk fat production (28 vs. 5%). Finally, the lack of variation in PTA for the tested animals might be because the 91 sires

TABLE 2. Least squares means (LSM) of pattern[1] effects for GH-*Taq*I and GH-*Msp*I on PTA for milk production and conformation traits and indexes of 91 Italian Holstein-Friesian bulls.

		Production										Conformation					
		Milk		Fat		Protein		Fat		Protein		FCS[3]		ICM[4]		ILQM	
Pattern	Bulls[2]	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
	(%)	(kg)						(%)									
GH-*Taq*I																	
AA	70.3	779.5	25	28.23	0.965	26.77	0.78	–0.0025	0.011	0.0365	0.005	0.413	0.0425	0.679	0.143	1695	48
AB	29.7	834	38	28.22	1.48	27.00	1.20	–0.0195	0.016	0.0245	0.007	0.437	0.065	0.620	0.219	1643	74
GH-*Msp*I																	
ABCD	34.1	801	38.5	29.18	1.51	27.66	1.27	0.002	0.017	0.042	0.0075	0.404	0.074	0.653	0.248	1764	79
BCD	65.9	800	26.5	27.17	1.04	26.14	0.875	–0.017	0.012	0.026	0.0055	0.444	0.0495	0.760	0.171	1629	55
$P > F$		NS[5]		NS		NS		NS		NS		NS		NS		NS	

[1]Generated fragments using *Taq*I or *Msp*I restriction enzymes on the growth hormone (GH) gene.

[2]Percentage of bulls.

[3]PTA for final conformation score.

[4]ICM = Genetic index combining linear conformation traits; ILQM = genetic index combining PTA for milk, fat, and protein production and linear conformation traits.

[5]$P \geq 0.05$.

TABLE 3. Least squares means of GHR-*Taq*I pattern[1] effects on PTA for milk production and conformation traits and indexes of 91 Italian Holstein-Friesian bulls.

		Production										Conformation					
		Milk		Fat		Protein		Fat		Protein		FCS[3]		ICM[4]		ILQM	
Pattern	Bulls[2]	LSM	SE	LSM	LE	LSM	SE	LSM	LE	LSM	SE	LSM	LE	LSM	SE	LSM	LE
	(%)	(kg)						(%)									
AB	8.8	797.5	69.5	29.21	2.73	25.78^{ac}	0.20	0.0055	0.031	0.023	0.013	0.426	0.124	0.843^{a}	0.398	1631^{b}	133
ABC	4.4	739	106	25.83	4.18	25.83	3.36	–0.0085	0.048	0.045	0.019	0.123	0.190	–0.950^{bc}	0.608	1374^{b}	203
ABCD	2.2	921^{ac}	129.5	30.00	5.12	32.00	4.12	–0.0325	0.059	0.047	0.024	0.675^{a}	0.232	0.485	0.744	1969^{ab}	248
ABD	3.2	959.5^{ac}	129.5	32.00	5.12	35.00^{ab}	4.12	–0.025	0.059	0.065	0.024	0.007^{b}	0.232	–0.890^{cd}	0.744	1958^{ab}	248
BC	29.7	834^{ac}	37.5	29.77	1.48	26.73^{ac}	1.19	–0.002	0.017	0.021^{a}	0.007	0.429	0.067	1.026^{a}	0.215	1703^{ab}	72
BCD	33.0	775^{a}	35.5	26.98	1.39	26.32^{ac}	1.12	–0.011	0.016	0.036^{a}	0.006	0.419	0.063	0.728^{ab}	0.203	1672^{b}	68
BCEF	9.9	793	65	26.37	2.56	25.38^{c}	2.06	–0.024	0.029	0.020^{a}	0.012	0.458	0.116	0.616^{ad}	0.372	1535^{b}	124
CD	6.6	595^{b}	82	23.50	3.24	25.00^{c}	2.61	0.019	0.037	0.084^{b}	0.015	0.366	0.147	0.336	0.471	1705^{ab}	157
CDEF	2.2	1046^{c}	129.5	32.75	5.12	35.25^{b}	4.12	–0.0465	0.059	0.042	0.024	0.490	0.232	0.885	0.744	2185^{a}	248
$P > F$		NS		NS		NS		NS		**		NS		NS		†	

[a,b,c,d]Column means with different superscripts differ ($P < 0.05$).

[1]Generated fragments using *Taq*I restriction enzyme on the growth hormone receptor (GHR) gene.

[2]Percentage of bulls.

[3]PTA for final conformation score.

[4]ICM = Genetic index combining PTA for linear conformation traits; ILQM = genetic index combining PTA for milk, fat, and protein production and linear conformation traits.

†$P < 0.10$. **$P < 0.01$.

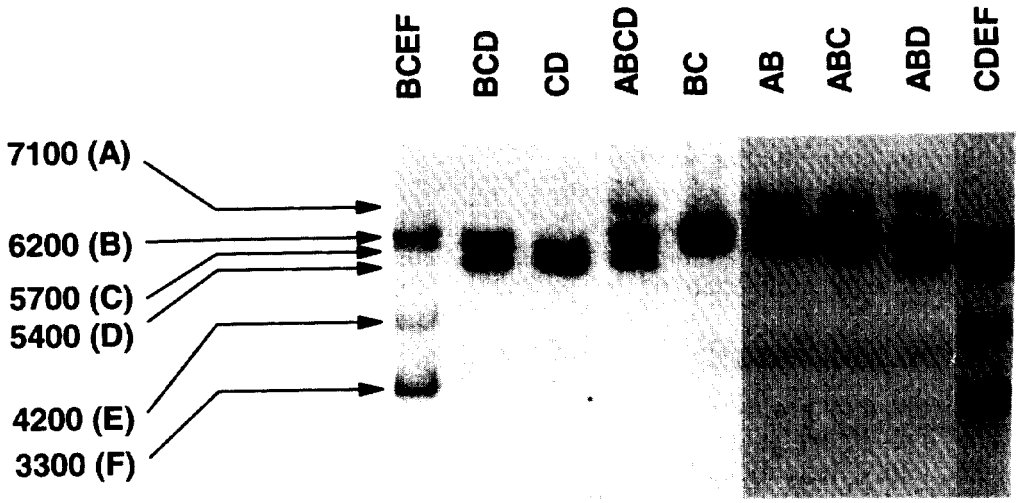

Figure 2. The generated patterns using the restriction enzyme *Taq*I on the growth hormone receptor gene observed in 91 Holstein-Friesian Bulls. The sizes of digested fragments are on the left, and the patterns are at the top. Fragment length (in base) was estimated relative to the DNA size markers λ*Hin*dIII and φX174 DNA/*Hae*III fragments.

were all positively tested bulls, which could limit the detection of a substitution effect. Also, in further studies, these methods need to be extended to less selected population (e.g., all bulls that were tested and have first results of progeny testing).

The Leu-Val polymorphism at the bovine GH gene has been investigated by other studies; using polymerase chain reaction and the restriction enzyme *Alu*I, Zhang et al. (38) revealed two alleles that were responsible for alternative forms of bovine GH with a Leu or Val residue at position 127. Those researchers (38) observed substantial variation of these allelic frequencies among eight evaluated breeds; mean frequency of the B allele in beef breeds was three times higher than expression in the Holstein breed. However, in a similar study of Holstein cows (24), differences in frequencies of these alleles were not significant between a control line and a line selected for milk production. This GH Leu-Val polymorphism was also investigated in cattle by Lucy et al. (26) and Schlee et al. (32), who did not observe any significant effect on milk production traits.

The PTA for milk production of bulls showing the GHR-*Taq*I CD pattern were significantly lower than the PTA of bulls with the patterns ABCD, ABD, BC, BCD, or CDEF (Table 3). However, the group of CD bulls had significantly higher mean (±SE) PTA of milk protein content ($P < 0.005$) by 0.063 ± 0.017%, 0.048 ± 0.017%, and 0.064 ± 0.020% than BC, BCD, and BCEF bulls, respectively. The negative effect of CD on milk production and its positive effect on milk protein percentage might be explained by the negative genetic correlation between these two traits. The least squares mean of PTA for milk production of bulls exhibiting the relatively frequent (33%) GHR-*Taq*I BCD pattern was significantly inferior ($P < 0.05$) to that observed for the low frequency (2.2%) CDEF pattern; the difference was 271 ± 135 kg of milk. For protein production, PTA were higher for bulls with GHR-*Taq*I CDEF than for bulls with AB, BC, BCD, BCEF, or CD ($P < 0.05$). Also, the least squares mean of the selection criterion index, ILQM, was significantly higher in bulls with the CDEF pattern group than for bulls with the AB, ABC, BCD, or BCEF groups, probably because of the favorable influence of CDEF on protein production. For conformation traits, the effects of GHR-*Taq*I RFLP on the final conformation score or the ICM index were not significant.

Because of the high polymorphism of GHR-*Taq*I, our statistical analysis was limited by the low number of the bulls in some pattern groups, especially for the interesting CD and CDEF groups, as in the study by Lucy et al. (26) of the Leu-Val GH polymorphism. A different experimental approach, such as the granddaughter design (37), with more animals is necessary to obtain more definite results and conclusions.

CONCLUSIONS

Three interesting findings resulted from the analysis of the relationship of GHR-*Taq*I RFLP to milk traits: 1) the positive effect of the low frequency CDEF pattern on ILQM, the index used as the selec-

tion criterion for the Italian Holstein-Friesian cattle population, 2) the higher PTA for milk production for cattle with this pattern than with the more frequent BCD, and 3) the favorable influence of CD on PTA for protein content.

These results indicate that further studies are needed at the DNA level to obtain more definite conclusions concerning the molecular events underlying the GHR-*Taq*I RFLP as well as its effects, direct or via a linked QTL, on milk production traits.

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