SYNERGISM BETWEEN GENETIC MARKERS IN THE GROWTH HORMONE AND GROWTH HORMONE RECEPTOR GENES IN INFLUENCING MILK RELATED TRAITS IN HOLSTEINS

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SUMMARY

A polymerase chain reaction (PCR) method was used to amplify a 345 bp region of intron 3 of the growth hormone (GH) gene and a 836 bp fragment from the 5' flanking region of the GH-receptor (GHR) gene. Digestion of the 345 GH fragment revealed an *Msp*I polymorphism and digestion of the 836 bp fragment revealed an *Alu*I polymorphism. The GH and GHR polymorphism and their interactive effect on milk related traits in 128 Holsteins bulls were tested under a linear model. There was no effect of the *Msp*I GH polymorphism; however, the *Alu*I polymorphism in the GHR gene was associated with milk yield. The significant interaction of the GH/GHR genotypes for milk protein was due to synergistic effect of the two genotypes. This is an example of the dependence of the effect of an allele in one gene (GH) on the allele in another gene (GHR). The study also suggests these markers may be useful for selection at the DNA level.

Key words: growth hormone, growth hormone receptor, Holstein.

INTRODUCTION

The main goal of breeders is to select superior animals for breeding. Screening for favourable alleles for selection at the DNA level provides an ideal tool for marker assisted selection either as parameters incorporated into selection indices,or for selection of juveniles in two-stage selection programs. Studies on genetic markers have involved quantitative trait loci (QTL) mapping, positional cloning or variants in candidate genes. Candidate genes are chosen for study on the basis of known relationship between biochemical or physiological processes and a trait, and tested as putative QTLs for the development of a genetic model. In dairy cattle, promising candidate genes for many traits are in the growth hormone (GH) axis. In dairy cows, GH has a profound effect on growth (Breir et al. 1991), lactation (Baumann 1992), and also modulates intermediary metabolism and other physiological processes such as aging (Copras et al. 1993), reproduction (Apa et al. 1994), and immune responsiveness (Blalock 1994). There are studies which suggest that genetic variants of the GH gene are associated with milk related traits in dairy cattle (Yao et al. 1996; Lucy et al. 1993; Hoj et al. 1993). Pertinent to the development of a genetic model for GH-axis is the role of GH-receptor (GHR). Growth hormone receptor induces mammary development, suggesting that GHR plays a central role in this process (Feldman et al. 1993). Growth hormone binds to proteins found in the cell cytoplasm, blood plasma and on cell surfaces, and the binding of GH to the membrane-bound receptor is the first step in the biological action of GH. There is evidence to suggest that the up-regulation of GHR may be central to the action of GH (Brier et al. 1991). In this communication, variants in the GH and GHR genes and

how they interact to influence milk related traits in Holsteins are studied.

MATERIALS AND METHODS

Semen samples were randomly collected from 128 progeny tested Holstein bulls. They were obtained from Centre d'insemination artificielle du Quebec (Saint Hyacinthe, Quebec, Canada), Western Ontario Breeders Inc. (Woodstock, Ontario, Canada) and United Breeders Inc. (Guelph, Ontario, Canada). Genomic DNA from semen was isolated as previously described (Zadworny and Kuhnlein 1990). Based on the published nucleotide sequence of the GH gene (Gordon et al. 1983) and the promoter region and exon 1 of the bovine GHR gene (Heap *et al* 1994) two pairs of oligonucleotide primers were synthesized to amplify two fragments. A 345 bp GH fragment which harbours an *Msp*I polymorphism was amplified from the GH gene, and the sequence of the forward and reverse primers, respectively were 5'-GGACAGAGATACTCCATCCAG-3' and 5'-AGATGCGAAGCAGCTCCAAGT-3'. An 836 bp GHR fragment containing a polymorphic *Alu*I site was amplified and the sequence of the forward and reverse primers, respectively were 5' TGCGTGCACAGCAGCTCAACC-3' and 5'-AGCAACCCCACTGCTGGGCAT-3' for the 836 bp fragment, and 5'-ATGCCCAGCAGTGGGGTTGCT-3'. PCR for both fragments were performed in a reaction volume of 25 ul using 100 ng of DNA, .25 uM of each primer, 1X PCR buffer [50 mM KCl, 1.5 mM $MgCl_2$ and 10 mM Tris-HCl (pH 9.0)], 5% deionized formamide, 160 uM dNTP and .625 units of *Thermus thermophilus* (Tth) DNA polymerase (Pharmacia, Baie D'Urfe, Quebec, Canada). Amplifications were carried out for 35 cycles at 92°C x 60s, 59°C x 80s and 72°C x 120s for the GH fragment and 92°C x 60s, 66°C x 80s and 72°C x 120s for the GHR fragment using a DNA thermal cycler (Perkin Elmer Cetus Corp., CT). For RFLP analysis, 10 ul of the 345 bp GH fragment and the 836 bp GHR fragment were digested with 5 units of *Msp*I and *Alu*I (Pharmacia), respectively. They were all digested at 37°C for at least 2 h. The digested DNA fragments were then separated by electrophoresis in 2% agarose gel in 1X TPE (90 mM Tris-phosphate, 2 mM EDTA). The gel was stained with ethidium bromide and visualized under UV light. Our analysis revealed that *Msp*I (-/-) PCR product exhibited two fragments of 286 bp and 59 bp. For the *Msp*I (+/+), the 286 bp fragment was cleaved into a 177- and a 109 bp fragments. The digested *Alu*I(-/-) PCR product exhibited three fragments of 747 bp, 75 bp and 14 bp (not detected on the gel). For the *Alu*I(+/+) PCR product, the 747 bp fragment was cleaved into two fragments of 602 and 145 bp.

The breeding values of the bulls for milk related traits (kg milk, kg fat and kg protein) were first estimated with the best linear unbiased procedure (BLUP) based on an animal model with relationship matrix. Preadjustments were made for effects of age and month of calving of daughters. The model included fixed effects of herd-year-season and age group of sires. The effect of GH and GHR genotypes on estimated breeding values for kg milk, kg fat and kg protein were then analyzed using least squares methods. As the breeding values are the best available estimates of the additive genotype of the bulls, no environmental effects were included in this model. The effect of birth-year of the bulls was included in the model to account for genetic trend. The model used was as follows:

$$Y_{ijkl} = u + Year_i + GH\text{-}type_j + GHR\text{-}type_k + GHxGHR\text{-}types_{jk} + e_{ijkl}$$

where Y_{ijkl} is the breeding value (kg milk, kg fat or kg protein) of the l^{th} bull; u is the least square estimate of the mean of the trait; $Year_i$ is the effect of the i^{th} birth year of the bull (genetic trend); GH-$type_j$ is the j^{th} GH genotype (j=1, 2, 3); GHR-$type_k$ is the k^{th} GHR genotype (k=1, 2, 3); GHxGHR-$types_{jk}$ is the interaction between GH and GHR genotypes; and e_{ijkl} is the random residual effect.

RESULTS AND DISCUSSION

Association of both GH and GHR genotypes and their interactive effect were analyzed using a linear model. The significance of the model and their components are represented in Table 1. The *Msp*I polymorphism did not show any significant association with any of the traits analyzed despite previously reported associations with milk related traits in different populations (Hoj et al. 1993; Lee et al. 1994). This may indicate that the genetic background of the population under study is important and may determine the type of associations obtained from a study. The *Alu*I GHR polymorphism was associated with milk yield. This polymorphism is located upstream of exon 1 in the 5' region (Aggrey et al. 1997). The 5' region of the GHR gene contains regulatory sequences which control the expression of GHR and interact with a large number of *cis*-acting and *trans*-acting factors (Heap et al. 1994). Modulation of the affinity of binding of any of these factors may affect GHR transcription and consequently its binding ability with GH.

Table 1. Significance of growth hormone (GH) and growth hormone receptor (GHR) genotypes and their interaction

	Significance (Pr>F)			
Trait	Model	GH	GHR	GH*GHR
EBV_Milk	0.0001	0.332	**0.038**	0.074
EBV_Fat	0.0001	0.570	0.528	0.488
EBV_Protein	0.0001	0.495	0.507	**0.019**

The combined effect of the marker genotypes of the two genes is shown in Table 2. For milk protein, the interaction between the growth hormone and growth hormone receptor genotypes was significant (P<0.019). The GH/GHR interaction for milk yield was approaching significance (P<0.07). Analysis of individual genotypic classes revealed that the interaction for the milk protein was due to a large effect of the GHR(-/-) genotype in a GH(+/-) background. The significant interaction of the GH/GHR genotypes for milk protein was due to synergistic effect of the two genotypes. This is an example of the dependence of the effect of an allele in one gene (GH) on the allele in another gene (GHR). Association studies between single genes or putative QTL and quantitative traits without taking into account the breeding structure and gene flow may be spurious because of non random association of gametes (Kennedy et al. 1992).

Table 2. Least square means for milk yield, fat content and protein content of Holstein bulls in dependence of growth hormone (GH) and growth hormone receptor (GHR) genotypes.

	Growth Hormone Genotypes							
	*Msp*I[+/+]			*Msp*I[+/-]			*Msp*I[-/-]	
	*Alu*I Growth Hormone Receptor Genotypes							
Trait	+/+	+/-	-/-	+/+	+/-	-/-	+/-	-/-
EBV_Milk	-1315	-1409	-1129	-1671[a]	-1189[a]	-113[b]	-669	-876
EBV_Fat	-36	-46	-42	-43	-45	-21	-	-14
EBV_Protein	-31[a]	-43[b]	-41	-49[a]	-39[a]	-17[b]	-21	-24

Differences in trait means of GHR genotypes within each GH genotypic class are indicated by a superscript ($P<0.005$).

Analysis based on real records of daughters of genotyped bulls would mean that the number in each subclass for fixed factors would be small. In addition, the data are not well connected, and fixed effects would have very poor estimates. The repeatability of the breeding values were high (95-99%) and most of the bulls used in this study were unrelated, and the animal model used in the estimation of the breeding values accounted for selection bias. However, a definitive conclusion would require segregation studies. This study indicates that the growth hormone and growth hormone receptor play a role in influencing milk related traits in Holsteins. The GH and GHR polymorphisms may serve as markers to select for in increasing milk yield and milk proteins at the DNA level.

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