ASSOCIATION OF GROWTH HORMONE LOCI WITH MILK PRODUCTION TRAITS IN HOLSTEIN BULLS

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SUMMARY

A pedigree analysis was used to investigate association of bovine growth hormone (bGH) loci with milk production traits in Holstein cattle. Three-hundred Holstein bulls were typed for three bGH loci: the polymorphism found in exon V; the polymorphism found in intron C; and the polymorphism found in the 3' region of the gene. Phenotypic data were daughter deviations for milk, fat, and protein yield, and fat and protein percentage. A two-step three-point linkage analysis was applied to the data, using pairs of bGH loci as markers linked to a putative biallelic quantitative trait locus (QTL). Parameters were estimated by maximum likelihood techniques. The estimated recombination fraction was close to 0 for milk yield and protein percentage, indicating physical linkage between a QTL affecting these traits and the bGH loci; however, LOD scores were less than one.

Keywords: quantitative trait loci, growth hormone, genetic markers, milk production.

INTRODUCTION

Bovine growth hormone (bGH) is part of a multi-gene family that contains prolactin and the placental lactogens and has been mapped by *in situ* hybridization to bovine chromosome 19 (Hediger *et al.* 1990). The gene is approximately 1,800 bp long with five exons (I-V) and four introns (A-D) (Woychik *et al.* 1982; Gordon *et al.* 1983). There have been several polymorphic regions reported in cattle. Lucy *et al.* (1991) reported a polymorphism in exon V that results in a change in amino acids of valine to leucine (Zhang *et al.* 1993a). Zhang *et al.* (1993b) reported a polymorphic site in intron C; and Unanian *et al.* (1994) reported a polymorphic site in the 3' region of the gene. These three polymorphisms were detected with PCR-RFLP techniques. Lagziel *et al.* (1995) found 14 different haplotypes for the entire bGH gene using single-stranded conformational polymorphic (SSCP) techniques.

The purpose of this study was to investigate association between the bGH loci and milk production traits in Holstein cattle by means of pedigree analysis.

MATERIALS AND METHODS

Data on 300 Holstein bulls, both young sires in progeny testing programs and active AI bulls, were used in the analysis. Bulls were typed for any combination of three bGH loci: the polymorphism found in exon V (GH427) with two alleles, A and B; the polymorphism found in intron C (GH891) with two alleles C and D; and the polymorphism found in the 3' region of the gene (GH441) with two alleles E and F (see Zhang *et al.* 1993a, 1993b, and Unanian *et al.* 1994

for protocols).

Phenotypic traits measured on bulls were daughter yield deviations (DYD) for milk, fat, and protein yield, and fat and protein percentage, obtained in the national evaluation from February 1997. Only bulls typed for at least two of three bGH loci and without missing phenotypic data were used. Pedigrees were constructed using data on bulls and their sires and dams. Some of the pedigrees involved 3 successive generations, since some bulls in the data appeared as sires or paternal grandsires of others. Dams were included in the analysis only to connect the pedigree members; no other information was available.

The analyses included linkage between bGH locus and a putative QTL affecting the trait, assuming that bGH loci act as markers linked to a QTL. The phenotypic measures were analyzed with a model that included the population mean, the effect of bGH genotype, a polygenic component and a random environmental component. The QTL was assumed to have two alleles, and three possible genotypes: 11, 12, and 22. The heritability of the polygenic component was assumed to be 0.25 for yield and 0.50 for percentage traits. Parameters were estimated for frequency of QTL alleles, recombination between the marker and QTL, genotypic means of the three QTL genotypes, and within-genotype standard deviations using maximum likelihood techniques. The likelihood function was maximized with respect to the unknown parameters using the Elston-Stewart algorithm (Elston and Stewart 1971). It was calculated for each pedigree separately, and then summed over all pedigrees. If marker and phenotypic data were missing, the likelihood calculation for those individuals was based on information available on parents or offspring, or derived from the average information from the population. Starting values for all analyses were 0.5 for allele frequency, 0.1 for recombination fraction, -1, 0, and 1 for the means of QTL genotypes 11, 12, and 22, respectively and 1 for standard deviation. Allele frequencies and recombination fractions were estimated within a parameter space between 0 and 1. When any of these parameters attained the boundary value, the value of the parameter was fixed at the boundary, and the maximization was continued to estimate other parameters until the likelihood was maximized. Computational aspects of likelihood calculation are given in PAP user manual (Hasstedt, 1994).

The analysis was conducted in two steps, using two adjacent bGH loci. In the first step, a linked, putative QTL is assumed to be located upstream from GH891-GH427. In the second step, a putative QTL was assumed to be linked to GH427-GH441 and located downstream from the bGH gene. All three polymorphic sites are completely linked. When the estimated recombination fraction was close to 0, the LOD score test was applied to test for linkage. The LOD score was defined as a log10 of the ratio of the likelihood between the model being tested and the model under the null hypothesis of free recombination between loci (θ=0.5).

RESULTS AND DISCUSSION

The data included 636 individuals assigned to 37 pedigrees. The pedigree size varied from 3 (a pair of parents and a progeny) to 226 (large multigenerational pedigrees with loops), with an average of 17.9 individuals per pedigree. Some of the pedigrees were extremely large due to dams

producing sons from two or more different sires. To reduce pedigree size and facilitate computation, the largest pedigrees were split into two or more smaller pedigrees by cutting loops (introducing a separate dam for each maternal half-brother). In this way, the pedigrees were simplified without loss of information. After cutting loops, the data included 645 individuals assigned to 45 pedigress. The average pedigree size was 14.3 individuals, with a minimum of 3 and a maximum of 79.

Means and standard deviations, respectively, for the milk production traits were 641 and 626 kg for milk yield, 20.7 and 26.3 kg for fat yield, 18.7 and 19.0 kg for protein yield, -.01 and .12% for fat percentage, and -.001 and .058% for protein percentage. Data were standardized by subtracting the mean and dividing by the standard deviation to enable comparison of differently scaled traits and to facilitate estimation of parameters of different magnitudes. The observed frequencies of each allele were: GH427 (n=288), A=.90, B=.10; GH891 (n=162), C=.90, D=.10; and GH441 (n=176), E=.83, F=.17.

Tables 1 and 2 contain results from linkage analyses assuming two different marker-QTL constellations: QTL-GH891-GH427, and GH427-GH441-QTL. Estimated parameters of the putative QTL had similar values irrespective of the configuration assumed. The frequency of the decreasing QTL allele ranged from .40 for milk yield to .57 for protein percentage. Genotypic means were in order 11<12<22 for all traits except protein percentage. For protein percentage, the heterozygous genotype at the QTL had a larger value than both homozygous genotypes. The effect of allele substitution, expressed as half the difference between two homozygous genotypes and given in standard deviation units, ranged from .57 for protein to 1.07 for fat percentage. The within-genotype standard deviation ranged from .68 for fat yield to .85 for milk yield.

Table 1: Linkage analysis for constellation QTL - GH891 - GH427.

Para-meter	Milk yield		Fat yield		Protein yield		Fat percentage		Protein percentage	
	E	S_E	E	S_E	E	S_E	E	S_E	E	S_E
p_1	.402	.091	.402	.094	.565	.137	.431	.078	.499	.085
$\hat{\theta}$	.000	--	.449	.358	.658	.191	.491	.284	.000	--
μ_{11}	-1.198	.254	-1.376	.230	-.873	.242	-.980	.273	-.964	.214
μ_{12}	.057	.159	-.366	.186	.030	.267	-.398	.200	.361	.179
μ_{22}	.368	.206	.772	.208	1.003	.274	.893	.197	.187	.241
SD	.853	.055	.681	.062	.733	.072	.720	.073	.801	.062
$\log_{10}L$	-286.9		-285.7		-285.1		-288.4		-283.87	
LOD:	0.168		***		***		***		0.332	

Table 2: Linkage analysis for constellation GH427 - GH441 - QTL.

Para-meter	Milk yield		Fat yield		Protein yield		Fat percentage		Protein percentage	
	E	S_E	E	S_E	E	S_E	E	S_E	E	S_E
p_1	.408	.090	.410	.094	.567	.130	.435	.079	.497	.083
$\hat{\theta}$	.000	--	.467	.331	.673	.190	.483	.311	.000	--
μ_{11}	-1.191	.249	-1.360	.227	-.874	.234	-.958	.279	-.976	.207
μ_{12}	.057	.158	-.353	.183	.033	.258	-.393	.203	.371	.180
μ_{22}	.391	.210	.783	.212	1.017	.264	.896	.201	.174	.241
SD	.850	.056	.681	.076	.730	.072	.724	.074	.797	.064
$\log_{10}L$	-305.9		-303.9		-303.3		-306.6		-302.8	
LOD	.154		***		***		***		0.41	

p_1 = QTL allele frequency; $\hat{\theta}$ = recombination fraction, μ_{11}, μ_{12}, μ_{22} = estimated means of QTL genotypes 11, 12, and 22, respectively; SD - within genotype standard deviation; E = estimated parameter value; S_E. standard error of the estimates; LOD = LOD score calculated as $\log_{10}$ [L($\hat{\theta}$)/L(½)]; *** = value not obtained.

The estimated recombination fraction was approximately 0.5 for fat and protein yield and fat percentage, indicating free recombination and lack of physical linkage between the bGH loci and a QTL. For milk yield and protein percentage the highest likelihood of the pedigrees was obtained assuming the recombination fraction of 0, and indicating close linkage between the bGH gene and the QTL. However, the obtained LOD scores were low and did not reach the conventional critical value of 3.

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