Structural variation around prolactin gene linked to quantitative traits in an elite Holstein sire family

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Summary. Digestion of genomic DNA with the restriction endonuclease AvaII disclosed a probable insertion/deletion of approximately 200 base pairs (bp) near the prolactin gene. Two alleles were apparent as three distinct hybridization patterns. These alleles were statistically associated with quantitative trait loci among sons of one elite Holstein sire family. The favorable genotype was correlated with the presence of a 1.15-kb hybridization band inherited from the sire when genomic DNA was probed with a full-length cDNA for prolactin. Pedigree estimates of genetic merit among genotypes were similar, differing by only 19.3 kg for milk in ancestor merit. Comparisons of genetic estimates for quantitative yield traits in offspring of this heterozygous sire showed significant (P<0.05) differences between homozygous genotypes for predicted difference milk (PDM), predicted difference dollars (PDS), cheese yield dollars, and protein dollars. The estimated differences between homozgyous genotypes for USDA Transmitting Abilities of PDM, PDS, Cheese Yield $ and Protein $ were 282.93 kg, $74.35, $48.58 and $53.67, respectively. However, the estimated breeding values from progeny ranged over 900 kg in transmitting ability for milk. Frequency of the favorable marker allele was estimated to be 0.231 in the elite cow population used as dams of sons. These results demonstrate the potential of molecular biological techniques to discriminate between individuals within a family and to predict breeding values for selection schemes.

Key words: Genetic marker - RFLP - Quantitative traits

Introduction

Linkages of genetic markers with quantitative trait loci (QTL) in breeding populations have been studied since the 1950s. Early studies dealt with blood group antigens and polymorphic criteria among body fluids. Although major loci affecting quantitative traits in farm animals have been found, linkage relationships among variants were rarely confirmed in subsequent studies. More recently, polymorphic markers in tomatoes (Tanksley et al. 1982), chromosomal markers for milk and blood protein variants in dairy cattle (Geldermann et al. 1985), marked chromosome sections affecting measured body characteristics in mice (Kluge and Geldermann 1982), and selection for immune responsiveness or disease resistance (Gavora and Spencer 1983) have been used to locate QTL.

Insertion/deletion or substitution events that alter the length of DNA fragments after restriction enzyme digestion have provided researchers with a large number of potential genetic markers to study linkage relationships with QTL. These restriction fragment length polymorphisms (RFLPs) may reflect differences in hormone products or simply provide a marker for another gene or group of genes on the same chromosome. RFLPs have been extensively used in human disease diagnosis and studies of modes of inheritance within families. As a result, a number of markers have been identified and applied in human linkage studies. The number of RFLPs linked to human diseases has increased tenfold between 1983 and 1987 (Watkins 1988) with an excess of 3000 markers being identified.

Utilization of marker genes within a breeding scheme requires linkage relationships of favorable chromosomal segments with detectable markers (Roberts and Smith 1982). The intentional crossing of lines homozygous at differing loci has been utilized to explore linkage relationships in the F2 offspring (Beckmann and Soller 1988). Although this method is well-adapted to plants and laboratory animals, it is impractical in field populations such as dairy cattle.
Theoretical strategies to utilize major genes and marker-assisted selection in plants and animals have been extensively developed (Beckmann and Soller 1983; Smith and Simpson 1986; Stam 1986; Soller and Beckmann 1988). The potential value of marker-assisted selection depends on the proportion of genetic variance associated with the marker loci, gene frequencies in the population, and the costs of testing programs (Roberts and Smith 1982; Geldermann et al. 1985). Since conveyance of chromosomes must be traceable in offspring, application of this method to dairy cattle breeding schemes must employ unique markers in parents or be planned to utilize only that proportion of matings where transfer of the marker is unambiguous in progeny (Beckmann and Soller 1988). The overall effects of unknown genes on the same chromosome as the marker may be neutral for a quantitative trait if animals of several families are involved in the mating scheme, since alleles may be linked differently in the various families. Geldermann (1975) and Stam (1986) have suggested the use of markers within families of a heterozygous parent as a way to follow passage of favorable or unfavorable chromosome segments. In addition, offspring from a heterozygous sire for a chosen marker would not be influenced by linkage disequilibria in the population, but would measure the effects of allele transfers on quantitative traits arising from substitution effects of paternal homologous chromosomes (Geldermann 1975).

Genes associated with mammary growth, development, and function are excellent candidates for linkage relationships with QTL. Using cDNA clones for bovine growth hormone and prolactin, four and three RFLP patterns, respectively, were detected within familial lines of Holstein bulls (Cowan et al. 1989). Development of statistical methods utilizing mixed-model methodology to detect major gene effects (Famula 1986; Hoeschele et al. 1988 a, b) has provided estimators of major gene effects in the presence of polygenic variation and unknown major genotypes and hypothesis testing procedures for these data. In the present study, sperm genomic DNA was digested with the restriction endonuclease AvaII, and the hybridization patterns detected with a full-length bovine prolactin cDNA clone were examined for linkage relationships with milk production traits within an elite Holstein sire family.

**Materials and methods**

This study examined the structural variation around the prolactin gene of 26 sons from one elite Holstein sire. In addition to sharing the same sire, several bulls also had the same maternal grandsire. One grandsire was common in the pedigrees of ten bulls, another sire produced three grandsons, and the remaining three pairs were represented by three additional grandsires. Seven of the half sibs had no other common ancestry. Estimated breeding values were obtained from United States Department of Agriculture Sire Summary January 1989, and represented yield information on a total of 1,650 granddaughters. The average USDA Repeatability was 71.2% for all genotypes.

Genomic DNA was extracted from spermatozoa as described by Camper et al. (1984). Ten micrograms of genomic DNA was digested with AvaII in accordance with the manufacturer's (New England Biolabs, Beverly/MA) recommendations. DNA fragments were separated by electrophoresis in 0.8–1.0% agarose gels and then blotted onto nylon membranes using the procedure described by Southern (1975). Filters were prehybridized in 5 × SSPE (1 × SSPE = 180 mM NaCl, 5 mM sodium phosphate, pH 7.4, and 0.5 mM EDTA), 0.4% SDS, 50% deionized formamide, 5 × Denhardt’s (1 × = 0.02% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone), and denatured herring sperm (50 µg/ml) at 42 °C for 6 h.

Bovine prolactin (pBPRL:72, Sasavage et al. 1982) cDNA radiolabeled by nick-translation (Rigby et al. 1977) was added with fresh hybridization solution, and the incubation continued for 36 h. Filters were washed twice with 2 × SSC (1 × SSC = 150 mM NaCl and 15 mM Na citrate, pH 7.0) at 65 °C for 15 min, followed by 2 × SSC and 0.1% SDS at 65 °C for 30 min, then once with 0.1 × SSC at 65 °C for 10 min. Filters were exposed to Kodak XAR-5 film with intensifying screens for 5 days at −80 °C.

In a preliminary study of 14 sons of one elite Holstein sire, DNA samples from each bull were digested with each of 11 restriction enzymes and hybridized with a cDNA clone for bovine prolactin. Digests from four restriction enzymes – AvaII, MspI, Rsal, and SacI – revealed variant hybridization patterns (Cowan et al. 1989). Digestion of DNA isolated from the sire of sons with each of the 11 restriction enzymes separately revealed an AvaII polymorphic pattern with two bands representing alternative alleles. Consequently, the structural variations around the prolactin gene within this sire family at these alleles were tested for linkage with QTL. Preliminary findings indicated a linkage relationship favoring those sons receiving one of the fragments from the sire. Subsequently, semen samples from additional sons were obtained and the DNA was examined. Included among the additional sons surveyed were those surviving progeny testing programs and thus this may have been a biased sample of all sons. This bias would create an underestimate of any gene substitution effects, since the sons with lowest genetic merit would have been eliminated.

**Statistical analysis**

The model proposed and tested included the genetic effects of major loci linked to marked chromosome fragments added to an underlying additive variance due to other genes (Dentine and Cowan 1990). The estimated variance within the homoyzous classes showed a positive relationship between the mean and variance. Data were transformed using natural logarithms to stabilize the variance within the homozogous classes of offspring. Estimates of D were obtained by using a specialized case of the general model for major loci presented by Hoeschele (1988a),

\[ Y_{ij} = \mu + \lambda_i \beta_j + g_i + e_{ij} \]

where \( Y_{ij} \) = yield trait based on first lactations of daughters of son \( i \) within RFLP genotype \( j = (1 \to 3) \); \( \mu \) = overall mean; \( \lambda_i \) = design matrix element representing the probability of a favorable linkage relationship; \( \beta_j \) = 1 for sons characterized as RFLP genotype AA \( (j = 1) \); \( \beta_j = 0 \) for sons characterized as RFLP genotype BB \( (j = 2) \); \( \lambda \) = maximum likelihood (ML) esti-