CANDIDATE GENES FOR PRODUCTION TRAITS IN DAIRY CATTLE

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SUMMARY

A $(CA)_n$ microsatellite (MS) in the 5' flank of the IGF-I gene and an RFLP in the growth hormone (GH) gene were tested for effects on production traits in Australian Holstein-Friesian cattle. Three alleles (127, 129 and 131 bp) at frequencies of 3.7%, 78.9% and 17.4% were found for the IGF-I MS and two alleles, Val^{127} and Leu^{127}, with frequencies of 18% and 82% were observed for the GH RFLP. The quantitative effect of the markers on milk production traits was estimated from 3,811 lactation records using an animal model. The IGF-I MS had no effect on milk production traits. The Leu^{127} allele of GH favoured higher production of milk, fat and protein and was dominant to Val^{127}.

Keywords: dairy cattle, milk production, candidate genes, IGF-I microsatellite, GH RFLP

INTRODUCTION

Systematic mapping of quantitative effects with respect to anonymous DNA markers in segregating pedigrees can theoretically identify all genes responsible for variation in economic traits. A possible short cut is to test candidate genes (Soller 1994).That is, DNA markers within or near genes whose products are known to be involved in relevant physiological processes and/or development are evaluated for effects on these traits. In the present study, a microsatellite marker in the bovine IGF-I gene, a candidate gene for milk production (Boer *et al.* 1990) and an RFLP marker in the bovine GH gene, also a candidate gene for milk production (Bauman *et al.* 1985), were studied to estimate the effect of these markers on production of milk, fat and protein in Australian dairy cattle.

MATERIALS AND METHODS

Animals. Four hundred and seventy-seven Holstein-Friesian cows were genotyped for the IGF-I microsatellite (IGF-I MS), using ^{32}P radioactive labelling. For the GH RFLP, 384 cows were genotyped for the previously described Val^{127}/Leu^{127} variants in exon 5 of the GH gene, using *Alu*I restriction enzyme (Chikuni *et al.* 1991). Also, three Holstein-Friesian AI sire half-sib families were genotyped for the IGF-I MS (total of 61 animals) and for the GH RFLP (total of 54 animals) with the same procedures. Primers for the IGF-I MS were from Kirkpatrick (1992) and for the GH RFLP were designed, using PCRPRIM software in ANGIS.

Datasets. Two separate datasets, namely lactation data and ABV data, were analysed to estimate the effect of the markers on production traits in cows. Lactation data included the records of cows which calved between 1986 and 1996 (years of calving) in 4 herds, belonging to the University of Sydney. Months of calving were classified into four groups, called seasons

of calving (December to February, March to May, June to August and September to November). Records for age of calving of more than 10 years were discarded because of scarcity of records in this group. This dataset consisted of yields of milk (l), fat (kg) and protein (kg), during a lactation period ranging between 60 and 400 days. Length of lactation (days) was also included in the data set. After editing, the dataset contained 3,811 records, including 1,372 records with IGF-I MS genotypes and 1,073 records with GH RFLP genotypes. ABV data included ABVs for milk, fat, protein and percentages of fat and protein which were separately analysed for cows and for AI sires.

Statistical model. The following univariate animal model was used to estimate the effect of candidate genes on production traits in cows:

$Y_{ijk} = \epsilon + G_i + bL + (HYS)_j + AC_k + A_l + e_{ijkl}$

where Y_{ijk} is the yield of milk (l), fat or protein (kg); ϵ is the general mean; b is the regression coefficient and L is the lactation length (days), fitted as a covariable; G_i is the effect of marker locus with 5 genotypes for the IGF-I MS and 3 genotypes for the GH RFLP, fitted as a fixed effect; $(HYS)_j$ is the effect of herd-year-season with 4x11x4 subclasses, fitted as a fixed effect; AC_k is the effect of age at calving (year) ranging between 2 and 10, fitted as a fixed effect; A_l is the additive polygenic effect of animal, fitted as a random effect; and e_{ijkl} is the random residual error. The multivariate Prediction and ESTimation (PEST) software (Groeneveld *et al.* 1992) was used. Averages of the traits for each genotype of the marker loci, adjusted for fixed effects and referred to as Best Linear Unbiased Estimates (BLUE), were compared by performing an F test. Additive genetic and residual variances required for these analyses were obtained from Derivative Free Restricted Maximum Likelihood (DFREML) analyses using an animal model (Meyer 1993). Additional analyses were also performed using more accurate estimates of variance components available from large data sets (Visscher and Goddard 1995). For ABV data, which have already been adjusted for fixed effects, a simple ANOVA technique was used to test the effect of each marker locus.

RESULTS AND DISCUSSION

Gene frequencies. Five genotypes, resulting from three alleles sized 127, 129 and 131, with frequencies of 3.7%, 78.9% and 17.4%, were observed for the IGF-I MS. For the GH RFLP, two alleles, namely Val^{127} and Leu^{127}, at frequencies of 18% and 82% were observed. The founder sires of the three sire families were homozygous for these markers. The estimated gene frequencies in the dam population of the sires were not significantly different from those in the cow population of the University herds.

Quantitative effects of the IGF-I MS. The 5 genotypes of the IGF-I MS were pairwise contrasted for yields of milk, fat and protein (data not shown). No significant differences were found between any of the genotypes for yields of milk and fat. For protein yield, genotypes 129/129 (188.70 kg) and 129/131 (188.58 kg) showed significantly higher BLUE values than genotype 131/131 (175.31 kg) ($P = 0.0066$ and 0.0086, respectively), suggesting a dominant effect of 129 relative to 131. However, there was no consistent pattern of superiority of 129

relative to 131 in genotypic combinations with allele 127. The results were consistent for analyses based on variance components estimated from the present study or taken from Visscher and Goddard (1995). No significant differences were observed between genotypes for ABVs for any of the traits.

Quantitative effects of the GH RFLP. Leu^{127}/Leu^{127} and Leu^{127}/Val^{127} were both significantly superior to Val^{127}/Val^{127} for lactation yields of milk, fat and protein (Table 1).

Table 1. BLUE values for lactation yields of milk (l), fat and protein (kg) for the GH RFLP genotypes[1]

	BLUE values			Difference between genotypes[2]		
Traits	L/L	L/V	V/V	L/L with L/V	L/L with V/V	L/V with V/V
Milk	5940.57[a]	5894.95[a]	5583.37[b]	45.6 (0.5)	357.2 (0.03)	311.6 (0.04)
Fat	262.65[a]	259.45[a]	236.18[b]	3.2 (0.3)	26.5 (0.003)	23.3 (0.01)
Protein	189.19[a]	187.78[a]	176.77[b]	1.4 (0.7)	12.4 (0.01)	11.2 (0.04)

[1] L/L = Leu^{127}/Leu^{127}, L/V = Leu^{127}/Val^{127}, V/V = Val^{127}/Val^{127}

[2] P values are in parenthesis

[a] and [b] Genotypes with the same letter are not significantly different

These two genotypes, Leu^{127}/Leu^{127} and Leu^{127}/Val^{127}, were not significantly different for any lactation yield. Superiority of the genotypes Leu^{127}/Leu^{127} and Leu^{127}/Val^{127} each compared to genotype Val^{127}/Val^{127}, calculated as arithmetic differences and as percentages were similar for yields of milk (357.2 l [6.4%] and 311.6 l [5.6%], respectively), fat (26.5 kg [11.2%] and 23.3 kg [9.9%], respectively) and protein (12.4 kg [7.0%] and 11.2 kg [6.3%], respectively). The results were consistent for the two different sources of variance components.

For the ABV data, in the cow population, genotypes Leu^{127}/Leu^{127} and Leu^{127}/Val^{127} were similar, both being significantly higher than Val^{127}/Val^{127} for ABVs for milk (398.8 kg, 366.2 kg and 153.6 kg respectively, P = 0.05), fat (18.3 kg, 15.7 kg and 8.0 kg, respectively, P = 0.01) and protein (11.2 kg, 10.7 kg and 6.0 kg, respectively, P = 0.05). No significant differences were found between genotypes for ABVs for percentages of fat and protein. Among the AI sires, there were only two genotypes (Leu127/Leu^{127} and Leu127/Val^{127}) available, which similar to their counterparts in the cow population, were not significantly different for any of the ABVs. Given that genotypes Leu^{127}/Leu^{127} and Leu^{127}/Val^{127} were similar and both showed higher performances than the Val^{127}/Val^{127} for all traits, the superior Leu^{127} allele is dominant to Val^{127}.

These results for the GH RFLP are consistent with the results of Lee *et al.* (1993) and Lucy *et al.* (1993), who reported that in American Holstein-Friesian cows, the Val^{127} allele is associated with low milk production. Schlee *et al.* (1994) also reported that in AI bulls of three cattle breeds in Germany, namely German Black and White, Bavarian and Tyrolean Brown and Simmental, the Leu^{127}/Leu^{127} genotype is associated with higher concentrations of

circulating growth hormone in different physiological conditions. Yao *et al.* (1996) reported that there were no significant differences between genotypes Leu^{127}/Leu^{127} and Leu^{127}/Val^{127} in Canadian Holstein bulls for milk yield, fat percent and protein yield.

In conclusion, there was no significant association between the IGF-I MS and production traits. By contrast, the dominant Leu^{127} allele at the GH locus favours higher production of milk, fat and protein. It can be concluded that this locus is a QTL or is in disequilibrium linkage with one or more tightly linked QTL. Either way, it could be a useful DNA marker for milk production traits in dairy cattle breeding programs in Australia.

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