Detection of Putative Loci Affecting Milk Production and Composition, Health, and Type Traits in a United States Holstein Population

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ABSTRACT

Quantitative trait loci affecting milk yield and composition, health, and type traits were studied for seven large grandsire families of US Holstein using the granddaughter design. The families were genotyped at 20 microsatellite markers on 15 chromosomes, and the effects of the marker alleles were analyzed for 28 traits (21 type traits, 5 milk yield and composition traits, somatic cell score, and productive herd life). Markers BM415 on chromosome 6 and BM6425 on chromosome 14 were associated with effects on protein percentage in a single grandsire family. The latter marker had a lower probability of being associated with changes in milk yield and fat percentage in the same family. Increases in productive herd life were associated with an allele at marker BM719 on chromosome 16 in one grandsire family.
(**Key words**: quantitative trait loci, microsatellite markers, milk yield traits, type traits)

Abbreviation key: **QTL** = quantitative trait loci.

INTRODUCTION

In 1923, Sax (17) described the first experiment designed to map genetic loci affecting quantitative trait loci (**QTL**). Since that time, the application of such experiments in livestock species has been limited by the number of genetic markers. However, with the discovery of several types of polymorphic DNA markers, a more comprehensive search for QTL is now possible. Since the publication of two genetic linkage maps of the bovine genome (4, 5), there have been several reports of potential linkages of QTL affecting health and milk production traits in dairy cattle with markers from the maps (1, 2, 8, 9, 14, 15, 20). To date, both the granddaughter and daughter designs (21) have been utilized to detect QTL in commercial populations of US, Dutch, and Israeli Holsteins (1, 2, 3, 8, 9, 14, 15, 20).

Our long-term goal is to identify QTL affecting milk production, health, and type traits in the US commercial Holstein population that will be useful in marker-assisted selection programs. Our first step toward achieving this objective was to conduct an initial genome-wide scan using approximately 90 highly polymorphic microsatellite markers located throughout the genome. Once QTL have been identified, additional markers will be selected to saturate the area for better localization of the QTL. Results from 16 markers have been reported previously (1, 2, 3) in which potential QTL for milk production and the type traits were identified in seven large Holstein families. The initial scan of the genome in these families is continuing. Here we report results from 20 additional microsatellite markers located on 15 bovine chromosomes.

MATERIALS AND METHODS

Source of Materials

Semen samples from seven large Holstein families were selected from the Dairy Bull DNA Repository (6), which is a collection of semen from 35 half-sib families in the granddaughter design. From 846 US Holstein bulls, DNA was isolated using a lysis and phenol-chloroform protocol as previously described (1).

January 1997 data for milk yield and composition, SCS, and productive herd life were provided by DHIA. Type trait data were provided by the Holstein Association USA (10). Composite indexes (udder, feet and legs, dairy capacity, and body form) were used as defined by the Holstein Association USA (10).

Microsatellite Markers

The 20 markers used in this study were selected from the USDA linkage map (5) and are listed in Table 1. Markers were selected based on their ability to coamplify with other markers and on the size of the

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TABLE 1. Microsatellite markers chosen for genotyping of all available sons of seven selected families from the Dairy Bull DNA Repository.

Chromosome	Locus	Alleles	Heterozygous grandsires	Sons genotyped	Informative	Sons
		(no.)				(%)
1	BM864	5	6	593	411	69.3
	BM1824	2	2	114	82	71.9
6	BM415	3	6	704	481	68.3
7	BM2607	2	4	384	240	62.5
9	BM2504	2	1	61	43	70.5
	BM757	3	4	388	244	62.9
11	BM304	5	5	527	449	85.2
	BM827	3	3	361	208	57.6
12	BM4028	5	7	734	640	87.2
14	BM4305	5	5	522	412	78.9
	BM6425	5	7	784	632	80.6
15	BM848	2	2	197	112	56.9
16	BM719	3	6	729	526	72.2
17	BM8125	4	6	734	533	72.6
19	BM6000	3	2	312	235	75.3
24	BM226	4	6	642	397	61.8
25	BMC3224	3	6	648	384	59.3
27	BM3507	4	3	274	167	60.9
28	BL25	4	5	578	437	75.6
	BP23	5	6	642	532	82.9

amplification product. Marker information, including polymerase chain reaction conditions, primer sequences, and linkage map locations, was reported by Bishop et al. (5) and is also available at the Meat Animal Research Center website (http://sol.marc.usda.gov). Polymerase chain reaction was performed using fluorescent primers as previously described (2). Amplification products were separated on a 5% Long Ranger (FMC, Rockland, ME) denaturing polyacrylamide gel and analyzed on an ABI automated DNA sequencer (model 377; ABI, Foster City, CA).

Statistical Analysis

The PTA or standardized PTA for the type traits and the daughter deviations for the health and milk production and composition traits were analyzed for marker effects within each heterozygous grandsire family using single-trait and multiple-trait analyses implemented by PROC MIXED and MANOVA of SAS PROC GLM (16). Within each sire family, the statistical model was

$$Y_{ijk} = M_{ij} + e_{ijk}$$

where Y_{ijk} = PTA or standardized PTA value of trait i for son k that inherited marker allele j, M_{ij} = effect of marker allele j on trait i, and e_{ijk} = random residual. Observations were weighted by the son reliability for trait i, which is proportional to the reciprocal of the variance of the daughter yield deviations. One-half of the dam PTA was subtracted from the daughter deviations of each son for milk, protein, and fat yields and percentages for SCS. For single-trait analysis, a significant marker effect was taken to indicate the presence of one or more closely linked QTL. For multiple-trait analysis, a significant marker effect was taken to indicate the sum of the pleiotropic effects of one QTL or a set of closely linked QTL, each affecting different traits.

Single-trait analysis was applied to each trait, and multiple-trait analysis was applied to each of the four groups of type traits (10): udder, body form, feet and legs, and dairy capacity. The udder group included six traits: fore udder attachment, rear udder height, rear udder width, udder depth, udder cleft, and front teat placement; the body form group included stature, body depth, rump angle, and thurl width; the feet and legs group included rear legs-side view, rear legs-rear view, foot angle, and feet and legs score; and the dairy capacity group included dairy form and strength. The multiple-trait analysis for each group of traits takes into account the variance-covariance structure among the traits and was conducted to detect various QTL that were associated with the traits in each group. The overall PTA value of all type traits from the genetic evaluation of the multiple-trait animal model (12) and the composite index for each group of traits were analyzed using the single-trait analysis. Single-trait analysis for each composite index shows the association of the index with the putative QTL and, therefore, has practical implications for marker-assisted selection if composite indexes are to be used. However, the analysis of a composite index uses just

one degree of freedom for one function of the traits; MANOVA analysis uses a combined test with degrees of freedom equal to the number of traits and summarizes the complete covariance matrix. Therefore, MANOVA provides a more complete test of the effect of an allele.

Canonical trait analyses were conducted as described by Weller et al. (22). The 17 linear type traits of standardized PTA were analyzed using a correlation matrix combined from several sources (11, 12, 19; T. J. Lawlor, 1998 personal communication). Because the combined matrix of genetic correlations was not positive definite, the genetic correlation of foot angle and feet and leg score was changed from 0.88 to 0.82, resulting in a valid correlation matrix. Milk, fat, protein, and SCS were analyzed using genetic (co)variances obtained from unpublished research (P. M. VanRaden, 1998, personal communication). As shown by Weller et al. (22), a reduction in number of traits that are analyzed is possible because a reduced number of traits accounted for a large fraction of the total variation. For the 17 conformation traits, the most important transformed trait accounted for over 31% of the variation, and 95 (99) % of the variation was accounted for by 10 (13) transformed traits. The situation was quite similar for the production traits with one transformed trait accounting for 69% of the variation and the remaining transformed traits contributing 17, 11, and 2% of the variation.

RESULTS AND DISCUSSION

We report the progress of a preliminary scan of the bovine genome to identify putative QTL affecting health, milk production and composition, and type

TABLE 2. Marker effects from single-trait analyses ($P < 0.01$).

Chromosome	Marker	Trait	Family code	Marker allele difference[1]	SE	*P*	n[2]
6	BM415	Protein percentage	9	0.05	0.01	0.00001	50
		Fat percentage	9	0.08	0.03	0.0069	50
		Body CI[3]	8	–0.86	0.29	0.0035	32
		Thurl width	8	–1.03	0.30	0.0005	32
7	BM2607	Fat yield	4	7.24	2.20	0.0012	73
		Feet and leg score	9	–0.82	0.31	0.0092	38
11	BM304	Fat yield	4	5.25	1.81	0.0040	102
		Fat percentage	4	0.05	0,02	0.0071	102
		Rear udder height	5	0.49	0.18	0.0067	110
12	BM4028	SCS	3	0.11	0.03	0.0020	102
		Herdlife	5	–0.93	0.32	0.0037	122
		Thurl width	12	1.71	0.60	0.0048	34
14	BM4305	Protein percentage	9	–0.03	0.01	0.0064	55
	BM6425	Milk yield	9	217.31	71.20	0.0024	47
		Protein percentage	9	–0.05	0.01	0.00002	47
		Fat percentage	9	–0.07	0.03	0.0083	47
		Rear legs-side view	4	0.63	0.24	0.0084	102
		Front teat placement	4	0.57	0.20	0.0047	102
16	BM719	Herdlife	3	1.29	0.39	0.0001	103
		Feet and legs CI	1	–0.40	0.15	0.0081	110
		Rear legs-side view	3	0.82	0.28	0.0032	90
		Foot angle	1	–0.53	0.20	0.0080	110
		Foot angle	8	0.88	0.33	0.0080	39
17	BM8125	Fat percentage	4	–0.07	0.02	0.0013	72
		Rear legs-side view	3	–0.63	0.24	0.0089	107
		Foot angle	8	–0.94	0.34	0.0066	39
		Udder cleft	5	–0.63	0.24	0.0094	73
19	BM6000	Rump angle	1	–0.72	0.24	0.0036	107
25	BMC3224	Protein percentage	9	0.04	0.02	0.0058	41
		Teat length	3	0.66	0.25	0.0097	82
27	BM3507	Feet and legs CI	8	–1.26	0.44	0.0049	36
		Rear legs-side view	8	1.85	0.67	0.0063	36

[1]Units of marker allele differences (smaller allele minus larger allele): milk, fat, or protein yield reported in kilograms; SCS adjusted to log base 2 of the concentration; percentage of protein and fat reported as percentage of (protein or fat yield/milk yield); and herd life reported as months of life, limited to 7 yr 10 mo of life per lactation.

[2]Number of informative sons with phenotypic data used in the analysis.

[3]Composite index.

TABLE 3. Marker effects from multiple-trait analysis of the type traits.

Chromosome	Marker	Trait group	Family code	*P*	n[1]
9	BM2504	Dairy capacity	9	0.0223	41
11	BM304	Body form	3	0.0370	111
		Dairy capacity	3	0.0413	111
12	BM4028	Body form	12	0.0030	34
		Dairy capacity	12	0.0070	34
		Udder	12	0.0356	34
		Udder	8	0.0261	51
14	BM6425	Dairy capacity	4	0.0399	102
		Udder	4	0.0103	102
		Udder	3	0.0385	112
17	BM8125	Body form	1	0.0297	148
25	BMC3224	Dairy capacity	3	0.0464	83
28	BP23	Dairy capacity	9	0.0266	51

[1]Number of informative sons with phenotypic data used in the analysis.

traits. Table 1 details the information content of the 20 microsatellite markers for which results are reported here.

Seven large Holstein grandsire families were selected from the Dairy Bull DNA Repository (6) for screening based on the number of sons available for genotyping. Based on the data from the 20 markers, on average, fewer than 5 of the 7 grandsires were heterozygous at each marker. The transmission of the grandsire alleles could be determined in 70.6% of sons from heterozygous sires. The number of informative sons is fewer than the total number genotyped because dam genotypes were unknown, and the transmission of the grandsire alleles could not be determined when son and grandsire had identical genotypes.

Data from 26 traits (19 type traits, 5 milk production and composition traits, SCS, and productive herd life) were used in single-trait analyses. Only results based on more than 30 observations are reported. Analyses were conducted within each grandsire family (Table 2) because associations between marker alleles and QTL alleles are expected to vary between families. Because of the large number of traits and tests involved, we have reported only effects for which $P < 0.01$. The analysis for the single traits produced 2392 tests of significance (92 informative families applied to 26 traits). Although not all tests are independent, comparisons of observed to expected significant effects indicate whether QTL effects are important relative to error. Twenty-four significant effects were expected by chance alone at $P < 0.01$, 3 at $P < 0.001$, and fewer than 1 at $P < 0.0001$. At these probability levels, 32, 4, and 2 significant effects were observed, respectively.

Based on these single-trait results, we have strong evidence of three QTL: two QTL affecting protein percentage and one QTL affecting productive herd life. The significant effect on protein percentage observed with marker BM415 in family 9 was not surprising because this marker lies close to the casein loci on chromosome 6, and there have been numerous reports of one or more QTL on this chromosome affecting the milk production and composition traits of different dairy populations (8, 9, 20). A comparison of the two grandsire alleles at this marker in family 9 reveals a relative decrease in milk yield ($P = 0.0109$) without a change in protein or fat yield, leading to an increase in the protein and fat percentages. The same effect has been observed on chromosome 6 for some families included in other studies (8, 9, 20).

On chromosome 14, we have evidence from one family of a QTL affecting milk yield and protein and fat percentages near marker BM6425. A comparison of the two grandsire alleles at this marker showed a relative increase in milk volume with no increase in protein or fat yield, thereby reducing protein and fat percentages. Recently, Ron et al. (15) reported an association between a marker on chromosome 14 and several of the milk production traits in a US Holstein population. In their study, marker CSSM66 had the greatest effect on fat percentage (significant at $P < 10^{-7}$). We may have detected the same QTL in our families, but the relative locations of CSSM66 and BM6425 on chromosome 14 are difficult to compare because each marker was mapped in different reference populations and no integrated map yet exists for this chromosome. However, based on physical assignments of a few markers from each map [(7); also, see http://locus.jouy.inra.fr], BM6425 and CSSM66 appear to be located at opposite ends of the chromosome. Therefore, we may be detecting a second QTL on chromosome 14 that is affecting milk production.

The third putative QTL we found is located on chromosome 16. Family 3 revealed a highly significant effect ($P = 0.000984$) on productive herd life at marker BM719. The herd life effect may be associated with effects on feet and leg traits for families 1, 3, and 8, which may be possible because the genetic correlation between productive herd life and foot angle is 0.23 (18). This report is the first of a potential QTL affecting productive herd life on chromosome 16.

Because many of the type traits are correlated, and within-family tests at many markers and traits generate numerous significance tests, we also used multivariate analysis of variance (Table 3) to analyze these data to reduce the number of significance tests to 368 (4 type groups applied to 92 marker-family combinations). Eighteen and 4 significant effects were expected by chance ($P < 0.05$ and $P < 0.01$, respectively), but we observed fewer significant effects and, therefore, lack conclusive evidence of associations between the type traits and the 20 markers used in this study. However, it is possible that QTL affecting the type traits exist on these 15 chromosomes but in regions further away from the markers described in this report.

Finally, as an alternative to the multivariate analysis of variance, canonical trait analysis was used to create traits that were approximately independent for analysis. Only results for the canonical traits with a cumulative proportion of variance of up to 95% are discussed. Results for the type trait, canonical trait analysis were consistent with the nontransformed traits. The first six canonical traits each had one significant marker in one family. The marker-family combinations were the same in five of those cases as combinations observed in the untransformed traits; BM757 in family 9 was the only addition with a significant effect observed for canonical trait 4. Additional marker effects were found in families for canonical traits 8 and 10, but significance was marginal ($P = 0.0064$ to 0.0090). Duplicate analyses for PTA for milk, fat, protein, and SCS were computed for comparison for two reasons. First, reliabilities could be different for each trait, and, therefore, it was not possible to use accuracy as a weight. Second, for consistency with the type trait canonical analysis, PTA were used. The unweighted single-trait analysis of PTA was consistent with those based on daughter yield deviations; BM2607 in family 5 had additional significance ($P = 0.0078$). The canonical trait analysis results for the production traits were similar to those for type traits, and the marker-family combinations found were similar to those in the original traits, except that BM415 in family 1 was found to be significant ($P = 0.0007$) for canonical trait 2.

CONCLUSIONS

This study found strong evidence of associations between three microsatellite markers and QTL for protein percentage and productive herd life in two US Holstein families. These results indicate that chromosomes 6 and 14 may have QTL affecting milk yield. Chromosome 16 may contain a QTL affecting productive herd life. Results from the set of 20 DNA markers tested here add to results for 16 markers reported by us previously (1, 2, 3) and to results for many other markers tested by other groups. These findings should help researchers identify the most useful markers available for QTL detection and, eventually, for marker-assisted selection for improvement of these traits.

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