Detection of Putative Loci Affecting Conformational Type Traits in an Elite Population of United States Holsteins Using Microsatellite Markers

M. S. ASHWELL,[1] Y. DA, P. M. VANRADEN, C. E. REXROAD, JR., and R. H. MILLER
Agricultural Research Service, USDA, Beltsville, MD 20705

ABSTRACT

Quantitative trait loci affecting conformational type traits were studied in seven large grandsire families of US Holsteins using the granddaughter design and 16 microsatellite markers on 10 chromosomes. The most significant marker effect was marker BM203 (chromosome 27) for dairy form in a single grandsire family. A multivariate analysis for dairy form and milk yield was also conducted, and the result was highly significant, indicating that a segregating quantitative trait locus or loci affecting dairy form and milk yield could exist near BM203 on chromosome 27. Marker BM1258 (chromosome 23) had a significant effect on udder depth. A multivariate analysis on udder depth and somatic cell score was conducted for markers 513 and BM1258, and both markers showed significant effects on these two traits, indicating that one or several quantitative trait loci affecting udder depth and mastitis might exist on chromosome 23. Marker BM4204 (chromosome 9) had a significant effect on foot angle and on the composite index of traits pertaining to feet and legs, indicating that one or several quantitative trait loci affecting traits pertaining to feet and legs might exist on chromosome 9. Selection on these markers could increase genetic progress within these families. (**Key words**: quantitative trait loci, microsatellite markers, type traits, dairy cattle)

Abbreviation key: **DBDR** = Dairy Bull DNA Repository, **PCR** = polymerase chain reaction, **QTL** = quantitative trait loci.

INTRODUCTION

Linear type traits measure biological and economic differences among dairy cows. At this time, 17 traits are evaluated by the Holstein Association using animal model procedures described by Misztal et al. (14). The Holstein Association currently has records from over 2 million cows (14). The traits are nearly normally distributed (18), and inheritance is assumed to be polygenic, although this assumption has not been investigated. Several estimates of heritabilities of type traits were reviewed (9, 10, 13, 15, 18, 19). Higher heritabilities imply more genetic variation and an increased probability that genes with substantial effect could be detected. Stature is generally reported to have the highest heritability among type traits (estimates range from 0.32 to 0.45), followed by body depth (0.15 to 0.37) and rump angle (0.23 to 0.33). Udder traits are generally intermediate: fore udder attachment (0.15 to 0.29), rear udder height (0.15 to 0.28), rear udder width (0.15 to 0.23), udder depth (0.20 to 0.28), udder cleft (0.10 to 0.24), teat length (0.26), and teat placement (0.18 to 0.26). Dairy form also has intermediate heritability (0.14 to 0.29). Type traits with low heritability are foot angle (0.07 to 0.15) and feet and leg score (0.17). Evaluations of linear traits receive direct economic emphasis in breed association indices and indirect emphasis in calculations of productive life; these evaluations are often displayed in advertising. Detection of genetic markers that are associated with the genes controlling these traits is useful in the initial steps to understand the relationship between the type traits and the genetic regulation of physiological characteristics and to identify markers that are potentially useful for marker-assisted selection for type traits. The purpose of this research was to identify genetic markers that were associated with type traits in an elite US Holstein population toward the eventual discovery of genes affecting type traits and marker-assisted selection for type traits.

MATERIALS AND METHODS

Source of Materials

Semen samples were selected from the Dairy Bull DNA Repository (**DBDR**) (5), located at the Univer-

Received April 14, 1997.
Accepted November 6, 1997.
[1]Corresponding author.

TABLE 1. Microsatellite markers genotyped for all available sons of seven selected Dairy Bull DNA Repository families.

Chromosome	Locus
2	BM4440
8	BM711
9	BM4204
14	BM302
18	BM2078
21	BM103
21	BM3413
22	BM3628
23	513
23	BM1258
23	BM1443
23	BM1818
23	BM1905
23	CYP21
26	BM4505
27	BM203

sity of Illinois (Urbana-Champaign). The DBDR is a collection of semen from 35 half-sib families in the granddaughter design (20). For a previous study (2), seven large families were selected from the DBDR based on the number of sons from which semen was available and the number of daughters with milk somatic cell information represented by each son (greater than 50 daughters per son). From approximately 900 US Holstein bulls, DNA was isolated using a lysis-phenol-chloroform protocol that has been described previously (2). The same seven families were used to identify potential quantitative trait loci (**QTL**) for the type traits in this report.

Type trait data were provided by Holstein Association USA (Brattleboro, VT). The type traits used in this study included values for PTA for type and standardized PTA values of fore udder attachment, rear udder height, rear udder width, udder depth, udder cleft, front teat placement, stature, body depth, rump angle, thurl width, rear legs-side view, rear legs-rear view, foot angle, feet and legs, dairy form and strength. Composite indexes (udder, feet and legs, dairy capacity, and body form) were used as defined by Holstein Association USA (9).

Microsatellite Markers

We report the results from 16 microsatellite markers located on 10 chromosomes (Table 1). Seven markers were chosen previously on their potential effects or estimated effects for SCS (1, 2). The remaining markers were chosen based on the availability of fluorescently tagged primers and the polymerase chain reaction (**PCR**) product size for use in a multiplex on an automated DNA sequencer (model 373; ABI, Foster City, CA). Marker information, including PCR annealing temperatures, primer sequences, and linkage map locations, was reported by Bishop et al. (4). All primers were identical to those described (4) with the exception of the BM2078 forward primer. A new forward primer was designed because the original PCR produced no amplification product using a fluorescently tagged forward primer. The BM2078 forward primer used in this study was 5′-CAGACTCTGAGCCCAAAAG-3′, making the PCR product 11 bp longer than the original PCR amplification product under the PCR conditions and annealing temperature described (4).

PCR and Gel Electrophoresis

The PCR was performed using either radioactive or fluorescent methods. Fifty nanograms of genomic DNA were placed into 96-well microtiter plates and amplified in the presence of 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* Tris·HCl, pH 9.0; 30 μM each of unlabeled dCTP, dGTP, and dTTP; 3.0 μM dATP, 0.1 μCi [α-^{32}P]dATP; 0.4 μM of each primer; and 0.35 units of *Taq* DNA polymerase in a total volume of 12 μl. Fluorescent PCR was performed as just described but with 30 μM each of unlabeled dCTP, dGTP, dTTP, and dATP and 0.4 μM of a fluorescently tagged forward primer and unlabeled reverse primer. The Hybaid Omnigene (Middlesex, Great Britain) thermal cycler protocol was as follows: 94°C for 3 min, 30 cycles of 1 min at 94°C, 1 min at the annealing temperature (4), 1 min at 72°C, and a final extension step of 5 min at 72°C. The MJ Research DNA Engine (Watertown, MA) thermal cycler protocol was similar except that each step was reduced from 1 min to 15 s. The PCR products were separated on a 6% denaturing polyacrylamide gel and exposed to film overnight (radioactive PCR) or analyzed on an ABI 373 Stretch Automated Sequencer (fluorescent PCR).

Statistical Analysis

Both PTA and standardized PTA values for type traits were analyzed for marker effects within each grandsire family using single-trait and multiple-trait analyses, implemented by ANOVA and MANOVA of the general linear models procedure of SAS (17). 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.

For single-trait analysis, a significant marker effect indicates the presence of one or more linked QTL. For multiple-trait analysis, a significant marker effect indicates the pleiotropic effect of a single QTL or the joint effects of multiple QTL affecting different traits. The single-trait analysis was applied to each trait, and the multiple-trait analysis was applied to each of the six groups of type traits: udder, body form, feet and legs, dairy capacity, udder-SCS, and milk-dairy form (only with marker BM203). The udder group includes six traits: fore udder attachment, rear udder height, rear udder width, udder depth, udder cleft, and front teat placement; the body form group includes stature, body depth, rump angle, and thurl width; the feet and legs group includes rear legs-side view, rear legs-rear view, foot angle, and feet and legs score; the dairy capacity group includes dairy form and strength; the udder-SCS includes udder depth and SCS; and the milk-dairy form includes daughter deviations for milk yield and dairy form. 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. Overall PTA value of all type traits from the multiple-trait animal model genetic evaluation (16) and 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 potential QTL and, therefore, has practical implications to marker-assisted selection if composite indices are to be used. However, the single-trait analysis on each composite index does not consider the statistical relationship among the traits and is not the best approach to detect QTL that are associated with the traits in each group. Multiple-trait analysis was also applied to dairy form and milk yield and to SCS and udder depth. Somatic cell score has been correlated to mastitis incidence (7), and deep udders have been shown to be more likely infected with organisms causing mastitis (3).

Because chromosome 23 was covered by six markers, interval mapping (11) was also conducted for all type traits using the ANIMAP programs (8).

RESULTS AND DISCUSSION

Table 1 details the 16 microsatellite markers used in this study. Based on the data from the 16 markers, approximately five of the seven grandsires (69%) were heterozygous at each marker. Data from 21 type traits were used in a single-trait analysis. Only results based on more than 30 observations are reported (Table 2). The analysis for the single traits produced 1617 significance tests. For $P < 0.01$, 16 significant effects were expected by chance, and 2 were expected at $P < 0.001$, if the traits were independent. At these probability values, 44 and 10 significant effects were observed, respectively. Based on the estimates of heritability, we might have expected the trait with the highest heritability (stature) to be more likely influenced by QTL. However, Table 2 reports only one significant effect related to stature (BM3413). In contrast, a trait with moderate heritability (fore udder attachment) had five significant effects at $P < 0.01$. The second highest number of significant effects, 4, was observed for udder depth, a trait that is highly correlated with fore udder attachment. Caution should be used, however, when these marker-QTL associations are being evaluated because of the large number of significance tests that were performed. Many associations may be due to chance, giving way to many false-positive claims if too lax a linkage standard is used. However, if too strict a guideline is used, many QTL may go unreported.

Lander and Kruglyak (12) calculated critical values to account for multiple testing over the entire genome to avoid large numbers of false-positive claims of linkage. Those researchers (12) state the need for genome-wide threshold values, where the probability value needed to be between 10^{-4} and 10^{-5} for significant linkage and 10^{-3} to 10^{-4} for suggestive linkage. Using these guidelines, we have identified three markers that show significant linkage; in family 8, linkage was between BM4204 and foot angle and between BM203 and dairy form, and, in family 12, linkage was between the udder traits and BM302.

Because chromosome 23 was covered by six markers, interval mapping (11) was conducted for all type traits using the ANIMAP programs (8). The most likely order of the markers is BM1443—BM1905—BM1818—CYP21—513—BM1258 with 3, 11, 11, 5 and 10 cM between the markers, respectively (4; http://sol.marc.usda.gov). In family 3 a potential QTL for teat length was detected 8 cM beyond BM1258, with a log of the odds score of 2.528, the largest we found, which is equivalent to a P value of approximately 0.001 (12). We did not detect this QTL using the single-marker approach; however, this QTL may be an artifact because the QTL was placed outside the region of the chromosome that was flanked by markers.

Because many of the type traits are correlated and because within-family tests for multiple markers and traits generate large numbers of statistical tests, we used MANOVA (17) to analyze the data (Table 3). This analysis produced 391 significance tests. A larger number of significant effects were observed

than would be expected by chance. If the traits were independent at $P < 0.05$, 20 significant effects would be expected by chance, but 35 were observed; at $P < 0.01$, 4 significant effects would be expected by chance, but 16 were observed; and, at $P < 0.001$, 0.4 significant effects would be expected by chance, but 2 were observed. The comparisons between the expected and observed numbers of significant results should be considered approximate because the reported results were limited to those with at least 30 observations. Thus, the observed number of significant results could have been underestimated, and, because some of the type traits have positive correlations, the observed number of significant results could have been overestimated. These two factors should partially cancel each other because they operate in opposite directions. Furthermore, the observed numbers of significant results generally were much larger than would be expected under the assumption of independent traits. Therefore, the approximate compar-

TABLE 2. Marker effects from single-trait analyses.

Chromosome	Marker	Trait	Family code	Marker allele difference	SE	*P*	n
2	BM4440	Dairy form	4	0.48	0.17	0.0044	113
		Fore attachment	4	0.48	0.18	0.0079	113
		Rear udder height	4	0.50	0.19	0.0098	113
		Rear udder width	4	0.64	0.18	0.0004	113
		CI[1] Dairy capacity	4	0.51	0.15	0.0007	113
		PTA for type	4	0.33	0.12	0.0068	113
8	BM711	Fore attachment	12	–0.86	0.32	0.0068	34
		Teat length	8	1.22	0.33	0.0003	34
9	BM4204	Rump angle	1	–0.66	0.20	0.0012	107
		Udder depth	8	1.04	0.40	0.0093	34
		Foot angle	8	–1.53	0.39	0.000099	34
		Rear legs side view	8	1.34	0.40	0.0011	34
		CI Feet and legs	8	–1.06	0.31	0.0007	34
		CI Dairy capacity	4	0.48	0.17	0.0055	101
14	BM302	Fore attachment	12	1.26	0.33	0.0002	32
		Front teat placement	12	1.42	0.35	0.000081	32
		Rear udder height	12	1.08	0.36	0.0029	32
		Rear udder width	12	0.98	0.35	0.0058	32
		Teat length	12	–1.01	0.36	0.0046	32
		Udder depth	12	1.04	0.39	0.0082	32
		CI Udder	12	1.10	0.28	0.0001	32
		PTA for type	12	0.59	0.23	0.0092	32
18	BM2078	Strength	12	0.77	0.28	0.0064	37
		Thurl width	12	0.80	0.28	0.0044	37
		Rear legs rear view	12	1.11	0.41	0.0067	37
21	BM103	Fore attachment	12	–0.82	0.31	0.0084	37
	BM3413	Stature	8	0.81	0.31	0.0084	36
		CI Dairy capacity	5	0.65	0.23	0.0057	67
22	BM3628	Rump angle	8	–0.79	0.28	0.0055	49
		Foot angle	8	0.99	0.34	0.0038	49
		Feet and leg score	8	0.81	0.29	0.0059	49
		CI Feet and legs	8	0.73	0.27	0.0080	49
23	BM1258	Fore attachment	1	0.46	0.15	0.0024	139
		Udder depth	1	0.61	0.18	0.0007	139
		CI Udder	1	0.38	0.13	0.0033	139
	BM1443	Thurl width	3	–0.44	0.16	0.0076	116
		Udder depth	9	–1.07	0.34	0.0019	57
	BM1905	Body depth	4	0.50	0.18	0.0067	89
		Strength	4	0.54	0.20	0.0057	89
		Foot angle	8	1.28	0.39	0.0012	45
	CYP21	Dairy form	3	–0.62	0.22	0.0047	80
		Rear legs rear view	1	0.62	0.22	0.0046	122
27	BM203	Dairy form	8	–1.27	0.30	0.000021	44
		CI Dairy capacity	8	–0.99	0.26	0.0002	44

[1]Composite index.

TABLE 3. Marker effects from multitrait analyses.

Chromosome	Marker	Family code	Group	*P*	n
2	BM4440	4	Dairy capacity	0.0034	113
			Udder	0.0017	113
8	BM711	8	Udder	0.0035	34
14	BM302	12	Udder	0.0052	32
		8	Udder-SCS	0.0060	42
22	BM3628	8	Feet and legs	0.0040	41
23	513	4	Udder-SCS	0.0021	80
23	BM1258	1	Udder	0.0066	139
			Udder-SCS	0.0045	136
23	BM1443	3	Body form	0.0087	116
23	BM1905	4	Dairy capacity	0.0025	89
23	CYP21	3	Dairy capacity	0.0086	80
26	BM4505	5	Dairy capacity	0.0054	111
27	BM203	8	Dairy capacity	0.00001	44
			Milk-dairy form	0.00001	44
		4	Udder	0.0045	80

isons should indicate that the observed significant results were not due to chance alone.

The multivariate analysis within family 8 revealed that the marker effect of BM203 (chromosome 27) on dairy capacity traits had the highest statistical significance (P = 0.00001; Table 3). Dairy capacity traits include dairy form and strength. The single-trait analysis showed a significant effect of BM203 on dairy form and the composite index for dairy capacity in the same family (Table 2) but not on strength. These analyses indicate that a QTL affecting dairy form may exist near BM203 on chromosome 27. A previous study (1) found that BM203 had a significant effect on milk yield. Therefore, a multivariate analysis for dairy form and milk yield was conducted for BM203. The significance levels for the multiple-trait analysis were about the same as for single-trait analyses. These results imply that a QTL or closely linked QTL may be affecting the milk yield and dairy form traits near BM203. The multivariate analyses showed that marker BM4440 (chromosome 2) also had a significant effect on dairy capacity (Table 3), and the single-trait analyses (Table 2) showed that the effect of BM4440 was on dairy form and on the composite index for dairy capacity, but not on strength. These analyses indicate QTL for dairy form may exist near BM203 on chromosome 27 and near BM4440 on chromosome 2.

Chromosome 23 showed interesting effects on body form and udder traits. The multivariate analysis (Table 3) showed that marker BM1258 had a significant effect on udder traits. Single-trait analysis (Table 2) showed the effect of BM1258 was mainly on udder depth. This result prompted a joint analysis of udder depth and SCS because deep udders may be more likely to be infected with organisms causing mastitis (3). Because data on mastitis incidence were not available, SCS was used because the two traits are correlated (7). A previous study (1) found a potential association between marker 513 and SCS; this marker is only about 10 cM from BM1258. Therefore, one QTL may affect both udder depth and SCS. The results showed that each of these two markers had a significant effect on both traits. The significance level for BM1258 was about the same as single-trait analysis; the significance level of marker 513 in family 4 was greatly improved from about P = 0.04 (2) to P = 0.0021 (Table 3). These analyses add more evidence that the region between markers 513 and BM1258 on chromosome 23 may be associated with mastitis resistance.

Although many significant effects were identified for several markers, many of these results are based on smaller sample sizes because only informative genotypes are used in the analyses. On average, the frequency of informative genotypes is about 77% in our data. Dentine and Cowan (6) have proposed a procedure to utilize noninformative genotypes. This approach would add more information to QTL detection and has been implemented for simulated data. This method is worth investigating for its application to QTL detection for which a large number of significance tests are often required. Another source of data reduction was due to missing data because some sons with marker genotype data did not have genetic evaluations. For those markers with insufficient sam-

ple sizes, more sons (currently unavailable) may be needed to confirm these results.

CONCLUSIONS

This study found strong evidence of associations between markers and QTL for dairy form and good evidence of these associations for udder depth and for feet and legs. These results indicate that chromosome 27 may have QTL with effects on dairy form, chromosome 9 may have QTL with effects on feet and legs traits, and chromosome 23 may have QTL with effects on udder depth. These findings provide important information for further studies in finding the genes affecting the type traits, identifying useful markers, and applying marker-assisted selection for type traits.

ACKNOWLEDGMENTS

The authors thank Tom Lawlor at the Holstein Association for generously providing the type trait data and Larry Shade for technical assistance.

REFERENCES

1 Ashwell, M. S., Y. Da, P. M. VanRaden, C. E. Rexroad, Jr., and R. H. Miller. 1997. Detection of loci affecting milk production and health traits in an elite US Holstein population using microsatellite markers. Anim. Genet. 28:216–222.

2 Ashwell, M. S., C. E. Rexroad, Jr., R. H. Miller, and P. M. VanRaden. 1996. Mapping economic trait loci for somatic cell score in Holstein cattle using microsatellite markers and selective genotyping. Anim. Genet. 27:235–242.

3 Bakken, G. 1981. Relationships between udder and teat morphology, mastitis, and milk production in Norwegian Red cattle. Acta Agric. Scand. 31:438–444.

4 Bishop, M. D., S. M. Kappes, J. W. Keele, R. T. Stone, S.L.F. Sunden, G. A. Hawkins, S. S. Toldo, R. Fries, M. D. Grosz, J. Yoo, and C. W. Beattie. 1994. A genetic linkage map for cattle. Genetics 136:619–639.

5 Da, Y., M. Ron, A. Yanai, M. Band, R. E. Everts, D. W. Heyen, J. I. Weller, G. R. Wiggans, and H. A. Lewin. 1994. The Dairy Bull DNA Repository: a resource for mapping quantitative trait loci. Proc. 5th World Congr. Genet. Appl. Livestock Prod., Guelph, ON, Canada 21:229–232.

6 Dentine, M. R., and C. M. Cowan. 1990. An analytical model for the estimation of chromosome substitution effects in the offspring of individuals heterozygous at a segregating marker locus. Theor. Appl. Genet. 79:775–780.

7 Emanuelson, E., B. Danell, and J. Philipsson. 1988. Genetic parameters for clinical mastitis, somatic cell counts and milk production estimated by multiple-trait restricted maximum likelihood. J. Dairy Sci. 71:467–476.

8 Georges, M., D. Nielsen, M. Mackinnon, A. Mishra, R. Okimoto, A. T. Pasquino, L. S. Sargeant, A. Sorensen, M. R. Steele, X. Zhao, J., E. Womack, and I. Hoeschele. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. Genetics 139:907–920.

9 Holstein Association of America. 1996. Holstein Type-Production Sire Summaries. Vol. 2. Holstein Assoc. Am., Brattleboro, VT.

10 Klei, L., E. J. Pollak, and R. L. Quaas. 1988. Genetic and environmental parameters associated with linearized type appraisal scores. J. Dairy Sci. 71:2744–2752.

11 Lander, E., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199.

12 Lander, E., and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat. Genet. 11:241–247.

13 Lawstuen, D. A., L. B. Hansen, and L. P. Johnson. 1987. Inheritance and relationships of linear type traits for age groups of Holsteins. J. Dairy Sci. 70:1027–1035.

14 Misztal, I., T. J. Lawlor, and T. H. Short. 1993. Implementation of single- and multiple-trait animal models for genetic evaluation of Holstein type traits. J. Dairy Sci. 76:1421–1432.

15 Misztal, I., T. J. Lawlor, T. H. Short, and P. M. VanRaden. 1992. Multiple-trait estimation of variance components of yield and type traits using an animal model. J. Dairy Sci. 75:544–551.

16 Misztal, I., K. Weigel, and T. J. Lawlor. 1995. Approximation of estimation of (co)variance components with multiple-trait restricted maximum likelihood by multiple diagonalization for more than one random effect. J. Dairy Sci. 78:1862–1872.

17 SAS/STAT® Software, Release 6.11. 1996. SAS Inst., Inc., Cary, NC.

18 Thompson, J. R., K. L. Lee, A. E. Freeman, and L. P. Johnson. 1983. Evaluation of a linearized type appraisal system for Holstein cattle. J. Dairy Sci. 66:325–331.

19 VanRaden, P. M., E. L. Jensen, T. J. Lawlor, and D. A. Funk. 1990. Prediction of transmitting abilities for Holstein type traits. J. Dairy Sci. 73:191–197.

20 Weller, J. I., Y. Kashi, and M. Soller. 1990. Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. J. Dairy Sci. 73:2525–2537.