Detection of Quantitative Trait Loci Affecting Milk Production Traits on Bovine Chromosome 6 in a Chinese Holstein Population by the Daughter Design

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ABSTRACT

Fourteen microsatellite markers with a coverage of 63.5 cM on bovine chromosome 6 were selected, and 26 sire families with 2,260 daughters were analyzed for mapping quantitative trait loci (QTL) affecting 5 milk production traits in a Chinese Holstein population. In the analyses across 26 families and within the largest significant families with a one-QTL model fitted, a QTL near BMS470 was detected that affected fat yield at the 5% experiment-wide significance level. When a 2-QTL model was fitted in the across-family analysis, it was found that there might exist 2 QTL affecting the 3 yield traits, although the exact or empirical thresholds for the significance testing were unknown. In all analyses, the results for milk yield and protein yield were generally consistent, which might have resulted from the same genetic background for milk and protein yield.

Key words: milk production trait, QTL mapping, *Bos taurus* autosome 6, daughter design

INTRODUCTION

According to the online combined QTL map of dairy cattle traits (http://www.vetsci.usyd.edu.au/reprogen/QTL_Map; published Oct 14, 2004), QTL affecting milk production traits may be carried on all 29 bovine autosomes. The QTL on BTA (*Bos taurus* autosome) 14 and BTA20 have been dissected to the genes acyl-CoA (or DGAT1; Grisart et al., 2001; Winter et al., 2002) and GHR (Blott et al., 2003), respectively. The effects associated with BTA6 have been reported since 1984 (McLean et al., 1984). The casein and albumin genes are located on BTA6, and the casein gene has been shown to have some effects on milk production traits (Velmalala et al., 1995; Ikonen et al., 2001; Prinzenberg et al., 2003). Most findings on BTA6 revealed a QTL

near marker BM143 (Spelman et al., 1996; Nadesalingam et al., 1998; Kühn et al., 1999; Velmalala et al., 1999; Ron et al., 2001; Olsen et al., 2002, 2004), which is about 35 cM away from the casein gene on the latest Meat Animal Research Center (MARC) map (Ihara et al., 2004). Several markers on BTA6 other than BM143 have also been reported to be closely linked with a QTL, such as BM415 (Ashwell et al., 1998; Mosig et al., 2001) and BP7 (Boichard, 1998). The multiple effects of QTL on BTA6 on milk production traits should be derived from the presence of multiple QTL or some pleiotropic QTL (Zhang et al., 1998; Ashwell et al., 2002; Freyer et al., 2003). Very recently, several candidate genes on BTA6 such as FAM13A1, PPARGC1A, and OPN were reported with potential effects on milk production traits (Cohen et al., 2004; Weikard et al., 2005; Schnabel et al., 2005).

Despite the fact that a large number of studies focusing on mapping QTL for milk production traits on BTA6 have been carried out to date, further studies are still needed to map these QTL more accurately and eventually to identify the genes themselves. For this purpose, 14 microsatellite markers were selected within the region that is believed to harbor QTL with significant effects on 5 milk production traits identified according to the previous studies. The 14 markers are spaced approximately evenly with an average interval of 5 cM except with higher density near the markers BM143 and BM415 according to the latest MARC map (Ihara et al., 2004). A population of Chinese Holstein cattle and a daughter design were used for QTL mapping in this region.

MATERIALS AND METHODS

Animals

Animals with a daughter design were selected from 9 Holstein cattle farms in Beijing. In the moderately sized populations for Chinese Holstein, potentially many more daughters were available with multiple records per cow. The Chinese Holstein originated from crosses of European Holstein-Friesian with Chinese

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Table 1. Sire families used in the analyses, total and informative number of daughters per sire family

No.	Sire	Daughters
1	2,085	86
2	2,086	129
3	2,087	96
4	2,088	152
5	2,089	113
6	2,090	74
7	2,091	54
8	2,093	94
9	2,094	118
10	2,097	66
11	2,098	35
12	2,099	67
13	2,100	70
14	2,101	102
15	2,102	130
16	2,103	47
17	2,104	83
18	2,105	39
19	2,106	125
20	2,107	45
21	2,110	193
22	2,113	60
23	2,114	76
24	2,115	47
25	2,117	57
26	2,118	102
Total		2,260

Yellow cattle about 65 yr ago. Since then, continuous introgression of foreign Holstein genes (live bulls, semen, and embryos), mainly from North America, have been conducted. Therefore, the current population has a close relationship with the North American Holstein. These 9 farms are the largest dairy cattle farms in Beijing with an average 305-d milk yield (**MY**) of about 8,500 kg. Regular and standard performance testing (DHI) have been carried out since 1999. After deleting the animals that were noninformative for any marker, animals whose marker genotype did not match with the paternal genotype for >3 marker loci, and the sire families with <30 daughters, 2,260 daughters of 26 sires remained in analyses with a range of 35 to 193 daughters per sire (Table 1). The 26 sires are sons or grandsons of the North American Holstein, and were born in the years between 1993 and 1996.

Marker Data

Fourteen microsatellite markers were selected from bovine chromosome 6. According to the latest MARC map reported at <http://www.marc.usda.gov> (published Jan. 20, 2005; Ihara et al., 2004), the 14 markers covered 63.5 cM; average interval length was about 5 cM (Table 2). Map distances between the 14 markers had been estimated with the program CRIMAP 2.4 (Green et al., 1990). The order of markers achieved was gener-

Table 2. Markers genotyped on chromosome 6, their relative map distances, number of alleles, and number of heterozygous sires for each marker

No.	Marker	Map distance (cM) ¹	Alleles	Heterozygous sires
1	BM1329	0.0	6	17
2	BMS2508	8.54	7	6
3	BMS1242	17.44	7	21
4	BM143	18.33	7	19
5	BMS518	23.57	4	13
6	BM4322	28.47	7	4
7	BMS470	32.0	4	18
8	ILSTS097	37.04	2	17
9	RM028	43.79	4	17
10	BM415	46.56	7	23
11	ILSTS035	51.87	9	12
12	ILSTS087	54.34	3	21
13	BMS2460	58.06	4	22
14	BP7	63.50	5	17

¹The latest Meat Animal Research Center map (Ihara et al., 2004).

ally consistent with the MARC map, but the total coverage was much longer. Because the MARC map has been commonly accepted and because it was shown that the differences in estimated recombination frequency did not bias the test for QTL or estimates of QTL effects (Haley and Knott, 1992), the MARC map was used in this study. The extracted DNA was always diluted and aliquoted to 96-well plates first. Then, PCR was performed on the GeneAmp PCR System 9600 or 9700 (PE, Applied Biosystems, Foster City, CA) under the following conditions: 20- μ L volume, 50 ng of genomic DNA, 1.3 μ M of each primer (5' ends of primers were labeled with fluorescein), 1.5 μ M of MgCl₂, 125 μ M of dNTPs, and 0.5 U of Taq polymerase. Annealing temperatures of PCR ranged from 55 to 61°C with 30 cycles of amplification.

The PCR products were run on the ABI 377 DNA sequencer (Applied Biosystems). Automated fragment analysis, size calling, and binning were then done by GeneScan v. 3.1 and Genotyper v. 2.5 (Applied Biosystems) to identify the alleles of each of the microsatellite loci. The numbers of alleles of the 14 markers found in the 26 families and the number of heterozygous sires for each marker are listed in Table 2.

Phenotypic Data

The official Beijing Holstein genetic evaluations are computed twice yearly at the Beijing Dairy Cattle Center. Milk yield, fat yield (**FY**), protein yield (**PY**), fat percentage (**FP**), and protein percentage (**PP**) over 305 d, preadjusted for calving age and month, are analyzed by a repeatability animal model consisting of herd-year-season effect, parity effect, permanent environmental effect, and animal effect (Zhang et al., 2000). The EBV

Table 3. Means, standard deviations, and minima and maxima of the EBV for the 5 milk production traits (2,365 cows)

Trait	Mean	SD	Minimum	Maximum
Milk yield, kg	363.77	464.51	-1,160.29	2,052.75
Fat yield, kg	14.32	16.59	-48.32	69.96
Protein yield, kg	11.14	14.16	-40.81	61.71
Fat percentage, %	0.015	0.194	-0.506	0.759
Protein percentage, %	-0.012	0.089	-0.371	0.327

from June 2004 evaluations were used as phenotypic data for this study. Means, standard deviations, and minimum and maximum values of the EBV for the 5 traits of the 2,260 cows are given in Table 3.

Statistical Methods for QTL Mapping

Using the marker data and the EBV of the 2,260 cows and their sires, linkage analysis was performed using the regression approach described by Knott et al. (1996) and the web-based software QTL Express (Seaton et al., 2001). The analyses were carried out first across all 26 families with both a 1- and 2-QTL model. With the significant families identified, within-family analyses were then conducted with a one-QTL model. The putative QTL were scanned every 1 cM within the region covered by the 14 markers. Two thousand five hundred permutations were used to estimate the 5 and 1% experiment-wise significance thresholds. The 95% confidence intervals of QTL positions were determined by bootstrapping. Three thousand bootstrap samples were generated from the data, and the shortest interval covering 95% of the samples was selected as the 95% confidence interval.

RESULTS

Analysis Across Families

The information contents are shown in Figure 1, calculated from variance of the conditional probabilities of inheriting a chromosomal region at each centimorgan as a proportion of the variance when true descent is known. The lower heterozygosity of marker BMS2508 (Table 2) and the larger distances between BMS2508 and the 2 adjacent markers resulted in lesser information in the first 2 marker brackets. At all other positions, the profile was generally flat with a small peak at each marker position, and all values were >0.65 .

In Figure 2, the test statistic (F -value) for one QTL at the given location vs. no QTL is shown across the area examined. The highest peaks for traits MY, FY, PY, and FP appeared at 32 cM, close to marker BMS470. The peak for PP was located at 58 cM, close to marker BMS2460. However, only the test statistic for FY ($F =$

1.90) was greater than the 5% experiment-wide threshold. A second peak ($F = 1.76$) appeared at position 15 cM, near marker BMS1242 for FY in Figure 2. It was also observed that the profiles for MY and PY fluctuated in generally the same way. The lengths of the 95% confidence intervals estimated from bootstrapping spanned from 51 to 59 cM for the 5 traits (Table 4).

For trait FY, for which a putative QTL with experiment-wide significance was identified, the QTL allele substitution effects were estimated for each family while fixing the QTL at the most likely position. Four sire families (families 2,085, 2,091, 2,102, and 2,103) were found to likely have significant QTL effect on FY (Table 4) based on the absolute t value with degrees of freedom equal to the number of informative daughters in the family. The average of the estimated absolute QTL substitution effect (8.26 ± 2.99 kg) in the 4 significant families was about one-third of the additive genetic standard deviation (28.44 kg, which was estimated from the phenotypic data). In Table 4, the families with t values >2 for the other 4 traits are also listed for reference. Note that family 2,102 is listed for all traits except FP, and for MY and PY the same 3 families were identified.

When a 2-QTL model was fitted, 2 tests were performed: Test 1, 2 QTL vs. 0 QTL; Test 2, 2 QTL vs. 1 QTL. In the latter case, the 2 tests were used to determine whether neither, one, or both positions explained a significant amount of the variance in the 2-QTL model. The tabulated probabilities corresponding to the calculated F -values are presented in Table 5. For the 3 yield traits, the probabilities for both tests were <0.05 ; for the 2 percentage traits, only probabilities for Test 1 were less than or close to 0.05. However, because a permutation test for the 2-QTL model is not feasible, it is hard to judge whether these probabilities reached a significant level.

Analysis Within Family

Within-family analyses were carried out for each of the 8 families listed in Table 4, but only results for families 2,102 and 2,110 are presented here, which are the largest among the 8 families. In fact, sires 2,102

Figure 1. Marker information content across 26 families (—), within family 2,102 (Δ), and within family 2,110 (---). Arrows indicate positions of markers.

and 2,110 are paternal half sibs, as sons of the U.S. Holstein bull 1919410, an internationally well-known bull because of its high performance for milk production trait. Within family 2,102, 130 informative daughters were included. The information within it (Figure 1) fluctuated more than that across 26 families, especially

in the interval between ILSTS035 and BMS2460. The peak locations of F -value profiles for the 5 traits were positioned at 32, 30, 29, 0, and 58 cM, respectively. The F -values at the peak locations for MY, FY, PY, and PP were near or above the 5% experiment-wide significance threshold (Table 6), but lower than the threshold ad-

Figure 2. Test statistic profiles (F -values) for 5 milk production traits [milk yield (---), fat yield ($\circ$), protein yield (—), fat percentage ($\times$) and protein percentage (Δ)] from the analysis across 26 families in a one-QTL model. Arrows indicate positions of markers, and the straight line indicates the 5% experiment-wide threshold for the fat yield trait.

Table 4. Quantitative trait loci position with the nearest marker, *F*-value, 5% experiment-wide threshold, 95% confidence interval (CI95), likelihood ratio (LR), and significant family concluded from analyses across 26 families in one-QTL model for the 5 milk production traits with QTL allele substitution effect estimated at the given QTL positions for each family combined with SE and absolute *t*-value [ABS(*t*)]

Trait ¹	Position (cM)	Nearest marker	<i>F</i> -value	Threshold	CI95 (cM)	Family	Effect	SE	ABS(<i>t</i>)
MY	32	BMS470	1.44	1.79	4–63	2,091	–198.01	75.55	2.62
						2,102	–122.52	59.33	2.07
						2,110	90.82	42.26	2.15
FY	33	BMS470	1.90	1.81	0–55	2,085	7.07	2.63	2.68
						2,091	–8.52	3.23	2.64
						2,102	–6.14	2.43	2.52
						2,103	11.31	3.67	3.08
PY	32	BMS470	1.41	1.78	5–63	2,091	–5.46	2.25	2.43
						2,102	–4.24	1.77	2.35
						2,110	2.69	1.26	2.14
FP	32	BMS470	1.61	1.86	0–54	2,085	0.07	0.03	2.07
						2,089	–0.07	0.03	2.22
						2,090	0.10	0.04	2.16
						2,105	0.11	0.05	2.17
PP	58	BMS2460	1.23	1.80	12–63	2,102	–0.04	0.02	2.29

¹MY = Milk yield, FY = fat yield, PY = protein yield, FP = fat percentage, and PP = protein percentage.

justed for multiple testing using Bonferroni adjustment when considering testing the 8 families independently. The test statistics for MY and PY varied generally in the same way, with an additional sharp peak in the region flanked by ILSTS035 and BMS2460 (Figure 3). The lengths of the 95% confidence intervals estimated spanned all along the interval studied and ranged from 56 to 63 cM.

Within family 2,110, 193 informative daughters were assayed. The information contents did not fluctuate so much as in Family 2,102, but with lower values in the first marker bracket (Figure 1). The QTL positions were all located at 32 cM for MY, FY, PY, and FP and at 19 cM for PP. The *F*-values for MY and FY were above the 5% experiment-wide significant threshold, but lower than the adjusted ones (Table 6). The *F*-values for FP and PP were rather low all along the region examined,

i.e., ≤ 1 . Again, *F*-value profiles for MY and PY fluctuated in the same manner (Figure 4). At the right side of the main peaks for the 2 traits, some subsidiary peaks were also present. The lengths of the 95% confidence intervals ranged from 35 to 61 cM.

DISCUSSION

With one-QTL model fitted in the analyses across 26 families, the most likely QTL positions for MY, FY, PY, and FP were all mapped at 32 cM, close to BMS470, and for PP, it was located at 58 cM, near BMS2460, although only the *F*-value of FY at the peak position was significant at the 5% experiment-wide level. These positions were also identified in the within-family analysis. Within family 2,102, the *F*-values for MY, FY, PY, and PP were close to or above the 5% experiment-wide

Table 5. Results concluded across 26 families with 2-QTL model fitted: QTL positions of the 2 QTL (A and B) with nearest markers in brackets and *F*-values and probabilities of the 2 tests (Test 1, 2 QTL vs. 0 QTL; Test 2, 2 QTL vs. 1 QTL)

Trait ²	Position		<i>F</i> -values		<i>P</i> ¹	
	A	B	Test 1	Test 2	Test 1	Test 2
	cM					
MY	36 (ILSTS097)	43 (RM028)	1.54	1.68	0.008	0.017
FY	36 (ILSTS097)	49 (BM415)	1.79	1.69	0.0005	0.016
PY	36 (ILSTS097)	43 (RM028)	1.48	1.58	0.015	0.031
FP	0 (BM1329)	29 (BM4322)	1.43	1.24	0.024	0.187
PP	32 (BMS470)	58 (BMS2460)	1.34	1.45	0.054	0.066

¹Tabulated probabilities corresponding to the calculated *F*-values using $F(52, 2,182)$ and $F(26, 2,182)$ for Test 1 and Test 2, respectively.

²MY = Milk yield, FY = fat yield, PY = protein yield, FP = fat percentage, PP = protein percentage.

Table 6. Quantitative trait loci position with the nearest marker, *F*-value, 5% experiment-wide threshold (Threshold), and 95% confidence intervals of QTL position (CI95) concluded from analyses within family 2,102 or family 2,110 in the one-QTL model for the 5 milk production traits

Family	Trait ¹	Position (cM)	Nearest marker	<i>F</i> -value	Threshold	Adj. Threshold ²	CI95 (cM)
2,102	MY	32	BMS470	5.44	6.30	10.55	0.0–57.0
	FY	30	BM4322/BMS470	6.32	7.20	12.08	0.0–56.0
	PY	29	BM4322	6.76	6.85	11.00	0.0–57.0
	FP	0	BM1329	3.83	7.20	11.26	0.0–63.0
	PP	58	BMS2460	7.38	6.62	11.14	0.0–63.0
2,110	MY	32	BMS470	6.54	6.18	10.54	18.5–63.0
	FY	32	BMS470	3.59	6.25	10.81	8.0–63.0
	PY	32	BMS470	6.96	6.45	11.11	28.0–63.0
	FP	32	BMS470	0.83	6.43	10.85	2.0–63.0
	PP	19	BM143	0.66	6.52	10.85	18.0–63.0

¹MY = milk yield, FY = fat yield, PY = protein yield, FP = fat percentage, and PP = protein percentage.

²Adj. Threshold = threshold adjusted for multiple testing.

thresholds, and the corresponding positions were almost the same as those observed in the analysis across 26 families. Within family 2,110, *F*-values for MY and PY were significant at the 5% experiment-wide level, and again, the corresponding positions were the same as those observed in the across-family analysis.

In the region near marker BMS470, some QTL affecting milk production traits have been reported in several previous studies. According to the latest MARC map (Ihara et al., 2004), BMS470 was located between TGLA37 and ILSTS097 with distances of 7.7 and 5.0 cM, respectively. Marker TGLA37 had been found to have effects on percentage traits (Zhang et al., 1998)

and yield traits (Kühn et al., 1996), and ILSTS097 on MY (Kühn et al., 1999; Moisio et al., 2000). Freyer et al. (2003) reported a significant pleiotropic QTL for FY and PY at the location bracketed by TGLA37 and FBN13. FBN13 was closely linked to BMS470 according to the high resolute radiation hybrid map built by Weikard et al. (2002), but has not been mapped in the MARC map.

Many previous studies reported a QTL for PP at position near BM143 (Spelman et al., 1996; Ron et al., 2001; Viitala et al., 2003) or near TGLA37 (Zhang et al., 1998; Ashwell et al., 2002). We were not able to confirm these results. In contrast, a possible QTL near BMS2460 was

Figure 3. Test statistic profiles (*F*-values) for 5 milk production traits [milk yield (---), fat yield (Δ), protein yield (—), fat percentage ($\times$) and protein percentage (Δ)] from the analysis within family 2,102 in a one-QTL model. Arrows indicate positions of markers.

Figure 4. Test statistic profiles (F -values) for 5 milk production traits [milk yield (---), fat yield (○), protein yield (—), fat percentage (×) and protein percentage (△)] from the analysis within family 2,110 in a one-QTL model. Arrows indicate positions of markers.

observed. In this study, a second test statistic peak for FY was observed near BMS1242 when analyzed across 26 families. Markers BMS1242 and BM143 were linked very closely with a distance of only 0.9 cM according to the latest MARC map (Table 2). Markers BM2460 is located between BM1236 and BP7 with distances of 3 and 4.5 cM, respectively, according to the latest MARC map (Ihara et al., 2004). There are some studies that revealed a QTL near BP7 (Ashwell et al., 1998; Boichard, 1998) or near BM 1236 (Ashwell et al., 2001). Therefore, it is very likely that a second QTL for PP exists in the region between BM1236 and BP7.

When a 2-QTL model was fitted, it was estimated that there were 2 QTL affecting each yield trait (Table 5). The 2 QTL near ILSTS097 and BM415 were affecting FY, and the 2 QTL near ILSTS097 and RM028 affecting MY and PY both. According to the literature review presented by Mosig et al. (2001), there were some reports for effects of these positions, but the case of 2 QTL coexisting for the 3 traits has never been reported.

The 95% confidence interval was estimated by using 3,000 bootstrap samples. In general, the sample frequency distributions were in accordance with the test statistic profiles, and the modes corresponded to the most likely QTL locations. The 95% confidence interval estimate covered very large intervals with a range of one-half to an entire chromosome area examined in this study. With a saturated genetic map, QTL position

resolution should be an inverse function of the QTL effect and the sample size (Darvasi and Soller, 1997). One reason for the large 95% confidence interval may be the relative small family sizes. Although the total sample size was rather large (2,260 daughters in total), the family sizes were limited, ranging from 35 to 195 daughters per sire. Further, the small QTL effect, the incompletely saturated map, and a possible substantial amount of linkage disequilibrium may also reduce the resolution.

In this study, all results for MY and PY were completely in accord, such as the significant families, the test statistic profiles, and the most likely QTL positions, no matter whether they were obtained from the across-family or within-family analysis, with a 1-QTL or a 2-QTL model fitted. This suggests that the 2 traits might have very similar genetic background. A pleiotropic QTL or 2 closely linked QTL may be the cause. However, because the 2 most likely positions obtained by the 2-QTL model analysis were in adjacent marker brackets, the estimates may not be reliable as point out by Witek et al. (1996).

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