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Genome screen for twinning rate QTL in four North American Holstein families

O. Cobanoglu*, P. J. Berger† and B. W. Kirkpatrick*

*Department of Animal Sciences, University of Wisconsin-Madison, Madison, WI 53706, USA. †Animal Science Department, Iowa State University, Ames, IA 50011, USA

Summary

The objective of this study was to identify twinning rate quantitative trait loci (QTL) by typing pooled samples in a preliminary screening followed by interval mapping to test QTL effects. Four elite North American Holstein half-sib sire families with high twinning rate predicted transmitting abilities (PTA) were used in this study. Chromosomes 5, 7, 19 and 23 were not genotyped as these chromosomes were scanned for QTL in these families in a previous study. DNA was extracted from phenotypically extreme sons in each sire family. Two pools were prepared from sons of sires in each phenotypic tail, two each for high and low PTA levels for twinning rates. Each pool contained DNA from 4 to 15% of all sons of the sire depending on family. A total of 268 fluorescently labelled microsatellite markers were tested for heterozygosity in sires. About 135–170 informative markers per family were genotyped using pooled DNA samples. Based on the preliminary evidence for potential twinning rate QTL from pooled typing, interval mapping was performed subsequently on 12 chromosomal regions by family combinations. Evidence of QTL for twinning rate was found in one family on BTA21 and 29 at a chromosome-wide $P < 0.05$ and on BTA8, 10 and 14 with a chromosome-wide $P < 0.01$.

Keywords dairy cattle, microsatellite, quantitative trait loci, selective DNA pooling, twinning rate.

Introduction

Twin calving is often considered an undesirable trait by cattle producers, and particularly by dairy producers. In general, there are many disadvantages that discourage attempts to select animals giving birth to more than one calf in the same lactation in dairy cattle. A number of studies showed that twin calving causes many detrimental effects especially on reproduction. Twinning reduces cow reproductive performance by increasing days open and service number per pregnancy during the subsequent parity as well as lessening prenatal survival of the calf (Cady & Van Vleck 1978; Nielen *et al.* 1989; Gregory *et al.* 1990). With regard to cow productivity, cows with high twinning rate have higher risk for dystocia, retained placenta, abortion, early embryonic loss and longer calving interval (Pfau *et al.* 1948; Markusfeld 1987; Nielen *et al.* 1989; Day *et al.* 1995). In spite of many detriments, twinning could be beneficial for beef producers under intensive management and feeding systems. In the beef industry, total production costs could be reduced 20–30% per unit beef returns by weaning twin calves (Guerra-Martinez *et al.* 1990).

Twinning rate is a difficult trait to improve by classical selection. In addition to being sex-limited, twinning rate has a low heritability. Estimates of heritability are typically on the order of 2–9% (Johanson *et al.* 2001). There is a high genetic correlation between twinning rate and ovulation rate ($r_G = 0.75 - 0.90$; Van Vleck *et al.* 1991; Gregory *et al.* 1997), which raises the possibility of indirect selection based on ovulation rate. Nonetheless, collection of multiple ovulation rate records would require significant time and cost. Identification of genetic markers linked with twinning rate QTL could provide a good alternative approach for genetic improvement of this trait.

Several studies have been conducted to identify ovulation rate and twinning rate QTL in different cattle populations. Putative ovulation rate QTL have been detected on BTA5 (Kappes *et al.* 2000), BTA7 (Blattman *et al.* 1996; Arias & Kirkpatrick 2004), BTA10 (Arias & Kirkpatrick 2004), BTA14 (Gonda *et al.* 2004), BTA19 (Arias & Kirkpatrick

Address for correspondence

B. W. Kirkpatrick, Department of Animal Sciences, University of Wisconsin-Madison, Madison, WI 53706, USA.
E-mail: bwkirkpa@wisc.edu

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2004), and BTA23 (Blattman *et al.* 1996) for ovulation rate using the USDA Meat Animal Research Center (MARC) twinning herd, a herd with substantial contribution from Holstein–Friesian and Norwegian Red breeds (Van Vleck & Gregory 1996). Lien *et al.* (2000) working with Norwegian cattle identified suggestive twinning rate QTL on BTA5, 7, 12 and 23. In a recent study with North American Holstein cattle, Cruickshank *et al.* (2004) found support for previously reported QTL on BTA5, 7, 19 and 23. However, there has been no search for twinning rate QTL on other chromosomal regions in Holstein cattle so far. The objective of this study was a genome-wide search for twinning rate QTL in Holstein families, excluding the chromosomal regions already scanned on BTA5, 7, 19 and 23. The approach used was typing of pooled DNA samples to identify regions of interest followed by interval mapping to test QTL significance.

Materials and methods

Animal resources and phenotypic data

Data for twinning rate from the North American Holstein breed were available through the National Association of Animal Breeders calving ease database. As previously described, over 1.3 million calving records collected from 1994 to 1998 were evaluated to estimate predicted transmitting ability (PTA) for twinning rate by threshold models (Johanson *et al.* 2001). A granddaughter design was applied to identify QTL (Weller *et al.* 1990). Twinning rate PTA on the underlying scale was used as phenotype in all QTL analyses. Information from granddaughters was used to estimate twinning rate PTA levels of sons. Sires, and sons of sires provided genotypic data. Four large, elite sire families were selected for the study, based on the sire having a twinning rate PTA above the population mean and a relatively large number of sons. A total of 63 sons from patriarch B, 56 from patriarch G, 63 from patriarch D and 55 from patriarch F were used in this study (Table 1). Semen samples were obtained primarily from the Cooperative Dairy DNA Repository (CDDR; Ashwell & Van Tassell 1999). Semen unavailable from CDDR was requested directly from AI studs and other sources. Genomic DNA was extracted from semen using a phenol/chloroform extraction method as in Cruickshank *et al.* (2004).

DNA pool formation

To conduct a genome wide search for twinning rate QTL, DNA pools were prepared from the sons of the sires in each phenotypic tail. For each sire, extreme and subextreme pools were created with respect to high and low PTA levels for twinning rates (Lipkin *et al.* 1998). Therefore, each sire was represented by four independent pools. Each pool contained 4–15% of all sons of the sire, which is equivalent to about 16–60% of sons being used in selective genotyping. Each pool consisted of 9 to15 animals depending on the sire. Some samples could not be used due to either lack of DNA or low amount of DNA. An equal amount of DNA from each selected individual was combined to form DNA pools.

Table 1 North American Holstein, paternal half-sib families used in this study.

Sire	Twinning rate (%)[1]	Total sons[2]	Sons used[3]	Calving records[4]
B	5.9	91	63	25–81
G	4.1	97	56	25–3632
D	5.1	147	63	26–202
F	4.8	263	55	26–1288

[1]Twinning rate, expected twinning rate of daughters.
[2]Number of sons with calculated twinning rate PTA.
[3]Number of sons with semen (DNA) available and obtained for use in this study.
[4]Range for number of daughter calving records used to estimate twinning rate PTA for each of the sons in the paternal half-sib sire family.

Genotyping of sires and DNA pools

A total of 268 fluorescently labelled cattle primers were used for microsatellite marker genotyping. PCR reactions were carried out in a PTC-100 thermal cycler (MJ Research, Waltham, MA, USA). Aliquots of 15 ng of genomic DNA were amplified in 12 μl volumes using 2.0–3.0 mM $MgCl_2$, 1.5X *Taq* buffer, 100 μM dNTP mix, 0.4 units of *Taq* DNA Polymerase (Promega, Madison, WI, USA), and 1–2 μM forward and reverse oligonucleotide primers. A 'touchdown' PCR protocol was used for genotyping (Don *et al.* 1991). Reactions were pre-heated to 95 °C for 3 min. After preliminary denaturation, reactions were cycled 12–18 times with denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s with temperature lowered 0.5 °C per cycle and extension at 72 °C for 40 s. After initial cycling, the next 20 cycles consisted of denaturation at 94 °C for 30 s, annealing at 52–56 °C for 30 s and extension at 72 °C for 40 s. Finally, the reaction was maintained at 72 °C for 20 min. After amplification, PCR products were electrophoresed on an Applied Biosystems 310 or 3100 genetic analyzer. ABI GeneScan and Genotyper (Applied Biosystems, Foster City, CA, USA) software were used to score each genotype. The CRI-MAP linkage analysis program was used to check for potential genotyping errors and determine the location of recombinations on each chromosome by utilizing the 'chrompic' option. (Green *et al.* 1990). Potentially erroneous genotypes were re-typed.

Marker allele band correction

An allele correction procedure that accounts for the presence of shadow bands from microsatellite alleles was

performed based on the method of Lipkin *et al.* (1998). Relative intensity (RI) of shadow bands had been estimated previously in our laboratory from multiple genotypes by dividing the shadow band area by the area of the main band. RI were calculated for the first three leading and the first trailing shadow band. Generic regression equations were developed previously by regressing RI of each shadow band on the number of repeat units (Gonda *et al.* 2004). Shadow-band effects were removed from the main bands by solving a set of simultaneous equations that describe expected shadow-band relative intensity. Total allele products were determined by adding the area of shadow-corrected alleles and the shadow bands derived from this allele. Finally, shadow-corrected sire allele frequencies within pools were estimated.

Statistical analysis

Strength of marker-QTL association for a given sire-marker combination was tested by *t*-test (Lipkin *et al.* 1998). The test was performed as follows:

$$t = (D_i/2)/\mathrm{SE}_{(\mathrm{DE}),}$$

where D_i is difference in sire allele frequency between high and low pools and $\mathrm{SE}_{(\mathrm{DE})}$ is an empirical estimate of allele frequency standard error. Standard deviations were calculated based on all possible pairwise differences between high and low pool frequency estimates.

Interval mapping

Interval mapping was performed to estimate and test putative QTL effects in particular chromosomal regions for specific families. A linear regression approach was used for interval mapping through the QTL express program (http://qtl.cap.ed.ac.uk; Seaton *et al.* 2002). The regression analysis was weighted by the inverse of the prediction error variance of each PTA value. The PTA values were weighted because number of calving records contributing to the PTA prediction for individual sires varied from 25 to 3600 and varied widely by family (Table 1). Genetic marker locations were obtained from the MARC-USDA bovine genome map (http://www.marc.usda.gov; Kappes *et al.* 1997). Chromosome-wide significance threshold levels were determined by permutation testing which involved shuffling twinning rate PTA values 1000 times randomly relative to the genotypic data (Churchill & Doerge 1994).

Results

A total of 504 *t*-tests were conducted in preliminary analysis of marker–QTL linkage. Only microsatellite markers with *t*-tests attaining a nominal *P*-value of < 0.01 are listed in Table 2. A total of 29 markers had provisional *P*-values of $P < 0.01$ based on the results from typing pooled samples. If all 504 tests were independent, only five results would have been expected to exceed this nominal significance level by chance alone. Based on these preliminary results from pooled typing, 14 chromosomal regions in specific families were chosen for further examination by interval mapping.

Table 2 Significant *t*-tests from selective DNA pooling.

Sire family	Marker	Chromosome	Position (cM)[1]	*t*-test
B	*BM7225*	3	102.4	2.93*
	BMS2116	27	55.4	3.87**
	BMS764	29	9.7	4.23***
G	*BMS2571*	4	66.3	5.04****
	BL37	5	50.2	2.85*
	BMS2072	8	58.8	4.23***
	BM711	8	83.6	4.43***
	BMS2349	10	22.1	−2.78*
	BMS2742	10	38.5	−4.07***
	BMS1716	11	47.7	2.95*
	BMS2047	11	73.8	3.32**
	IDVGA3	11	76.9	4.50***
	BL1103	11	90.9	6.20****
	BMS108	14	50.8	3.29*
	ADCY2	15	12.1	2.94*
	BMS745	19	15.9	3.31**
	BM8115	21	0.0	−6.91****
	BM1314	26	24.8	3.47**
	BMS2650	27	12.5	5.77****
	RM044	29	23.3	−3.99***
	URB011	29	55.6	−2.61*
D	*BMS2519*	2	101.5	3.05**
	ILSTS036	11	56.9	2.81*
	BMS1825	17	3.8	−3.53**
F	*TEXAN2*	2	22.5	−2.66*
	BM2808	2	59.9	−2.97*
	BMS462	16	93.2	−3.45**
	BL25	28	17.8	−3.17*

*$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$; ****$P < 0.00001$.
[1]Microsatellite positions were acquired from the bovine genome map compiled by the MARC-USDA (http://www.marc.usda.gov; Kappes *et al.* 1997).

Five of the 14 chromosomal regions examined by interval mapping showed evidence for an association with twinning rate (Table 3) at either a chromosome-wide $P < 0.05$ or $P < 0.01$. Results from interval mapping analysis in family G support the presence of QTL for twinning rate on BTA8, 10 and 14 at a chromosome-wide $P < 0.01$. (Fig. 1). Interval mapping results in the same family on BTA21 and BTA29 exceeded chromosome-wide $P < 0.05$ thresholds. In all other cases QTL were not supported by results from interval mapping at a chromosome-wide threshold of $P < 0.05$. Estimated QTL effects were large, on the order of one standard deviation of PTA; however, these estimates are likely overstatements of the true effect, given that they are

Table 3 Interval mapping results for four families.

Family	Chromosome	Markers (no.)	Position (cM)[1]	Max *F*-ratio	Effect ± SE[2]	Chromosome-wide *P*-value	Nominal *P*-value
B	27	3	45.5	0.96	0.029 ± 0.029	NS	0.333
B	29	3	26.5	1.16	0.027 ± 0.024	NS	0.286
G	8	6	116.7	12.05	0.155 ± 0.044	$P < 0.01$	0.001
G	10	5	41.0	11.17	0.105 ± 0.031	$P < 0.01$	0.001
G	11	8	82.7	3.54	0.064 ± 0.033	NS	0.065
G	14	5	67.9	14.45	0.119 ± 0.031	$P < 0.01$	0.001
G	21	3	2.0	7.18	0.105 ± 0.040	$P < 0.05$	0.010
G	27	3	46.5	0.21	0.016 ± 0.034	NS	0.649
G	29	4	63.3	8.80	0.098 ± 0.033	$P < 0.05$	0.004
D	11	8	67.7	1.85	0.036 ± 0.026	NS	0.179
D	17	3	12.8	1.67	0.045 ± 0.034	NS	0.201
F	2	4	68.5	4.93	0.094 ± 0.042	NS	0.031
F	16	3	62.4	2.38	0.085 ± 0.055	NS	0.129
F	28	3	20.8	1.12	0.058 ± 0.054	NS	0.280

NS, not significant.

[1]Distance from centromeric end of chromosome.

[2]Effects stated on the liability scale from the threshold model. An effect of 0.1 corresponds approximately to a change of 0.8% in twinning rate.

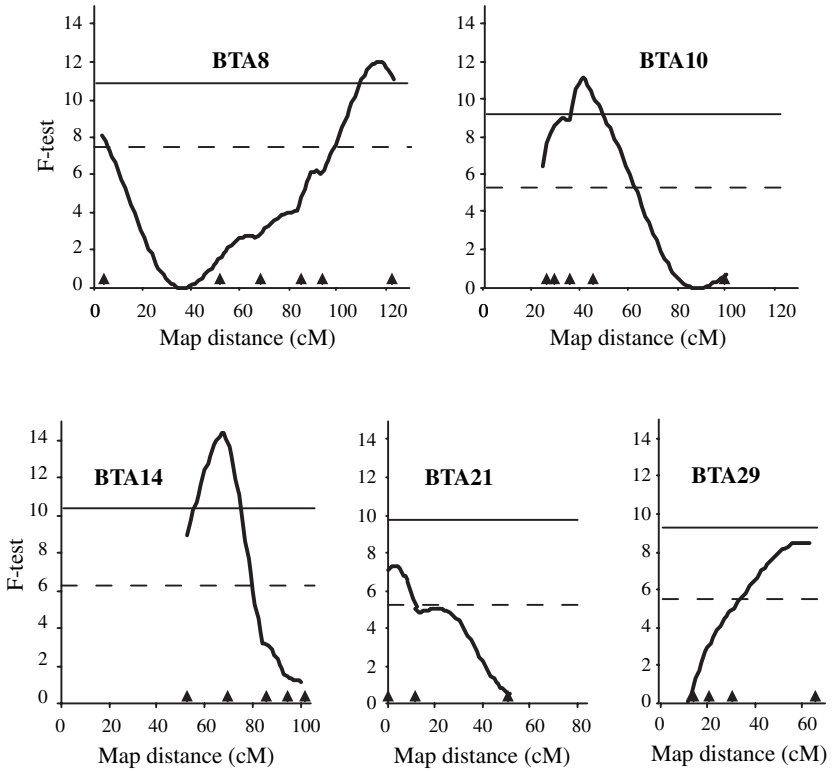

Figure 1 Plot of *F*-statistics from interval mapping analysis versus map distance on BTA8, 10, 14, 21 and 29 for family G. Arrows represent the location of markers genotyped (in order BTA8:*BMS1864*, *BM4006*, *BMS2072*,*IDVGA52*, *BM711*, *BMS836*; BTA10:-*BMS528*, *BMS2349*, *BRN*,*BMS2742*, *BMS614*; BTA14:*BMC1207*, *BMS108*, *BM2934*,*BMS2055*, *BL1036*; BTA21:*BM8115*, *RM151*, *TGLA337*; BTA29:*BMS764*, *BMS1787*, *BMS1600*, *BMS1948*). Horizontal dashed and solid lines represent empirical chromosome-wide $P < 0.05$ and $P < 0.01$ thresholds, respectively. Length of the *x*-axis corresponds to the length of the respective chromosomal linkage map (Kappes *et al.* 1997).

based on the least squares regression analysis of selectively genotyped data (Darvasi & Soller 1992).

Discussion

The selective DNA pooling method has been used in various studies (Mosig *et al.* 2001; Fisher & Spelman 2004; Gonda *et al.* 2004) to search for linkage between markers and QTL for production and reproduction traits as it reduces the amount of genotyping with minor loss of power (Darvasi & Soller 1994). Therefore, it is faster and less costly to identify potential QTL locations. Potential evidence for twinning rate QTL in more than 12 chromosomal regions in North American Holstein families was revealed using the selective

DNA pooling approach in this study. Given the modest family sizes and associated low power, selective DNA pooling was used here as a screening tool to identify chromosomal regions to examine with the more powerful interval mapping analysis.

This genome-wide search using typing of pooled samples and subsequent interval mapping revealed the strongest evidence for twinning rate QTL on BTA8, 10 and 14. The chromosome-wide $P < 0.01$ significance level surpassed in these three cases corresponds to a genome-wide suggestive significance level as proposed by Lander & Kruglyak (1995) based on the associated nominal *P*-values at the maximum *F*-value. Previous studies have also provided evidence for ovulation rate QTL on chromosomes 10 and 14 (Arias & Kirkpatrick 2004; Gonda *et al.* 2004). On the contrary, there are no previous reports of twinning rate or ovulation rate QTL on BTA8. Arias & Kirkpatrick (2004), using data from the USDA-MARC twinning herd, reported evidence of a BTA10 QTL that was more telomeric (about 75 cM) relative to the location reported here. However, Gonda *et al.* (2004) could not confirm the presence of QTL on BTA10 using related family material from the USDA-MARC herd. Gonda *et al.* (2004) did report a putative ovulation rate QTL on BTA14 whose location corresponds well with that reported here.

Regarding chromosomes examined by the interval mapping analysis, a total of 22 microsatellite markers on 12 chromosomal regions displayed preliminary evidence for twinning rate QTL with at least $P < 0.01$ based on selective DNA pooling. However, subsequent interval mapping analysis failed to support the existence of QTL for a majority of these regions. There are several possible explanations for this discrepancy. One possibility is that putative linkage between marker and QTL detected in analysis with pool typing might have been the result of sampling, e.g. sire-type alleles could have been differentially contributed by the dams to the high and low pools. Another item contributing to differences between pooled typing and interval mapping results is the equal contributions of sons to pools, but differential contribution to the weighted, interval mapping analysis. In some instances, low marker density and differential levels of informativeness for some chromosomal regions may have contributed to a loss of information in interval mapping. Another concern is that some *t*-value results may be inflated based on selective DNA pooling, owing to an exceptionally low estimated standard error of allele frequency difference. Some of the markers with significant *t*-values in preliminary screening, such as *BM7225* on BTA3 for sire family B and *BMS2571* on BTA4 for sire family G (Table 2), were not examined further by the interval mapping analysis for this reason because only a single marker on these chromosomes showed evidence for linkage and had a potentially inflated *t*-value. Instead of using individually estimated standard errors of pool allele frequency differences, use of an average standard error per family for *t*-test calculations could reduce this problem.

All significant interval mapping results were identified in one family (G). It seems unlikely that only one of the four sire families examined here would be segregating twinning rate QTL. One possible explanation for the increased power to detect QTL in this family relates to the weighted regression analysis. The preponderance of significant results in the G family reflects a case where animals with two of the three most extreme phenotypes also coincidentally had the greatest weights owing to much higher accuracies of PTA.

Although nine chromosomal regions examined by interval mapping failed to provide further evidence supporting the selective pooling results, selective DNA pooling reduced genotyping cost by up to 12-fold compared to individual genotyping. A genome-wide scan for twinning rate QTL was achieved in this study for four North American Holstein half-sib families. Five genomic regions displaying evidence for potential twinning rate QTL were detected by selective DNA pooling followed by interval mapping. These putative QTL require verification by replication in different cattle families.

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