FINE-MAPPING OF QTL AFFECTING PROTEIN PERCENT AND FAT PERCENT ON BTA6 IN A POPULAR U.S. HOLSTEIN FAMILY

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INTRODUCTION

Results from numerous genome-scans, including our own (Ashwell *et al*., 2001), have identified putative QTL affecting milk production, health and conformation in different dairy populations (e.g. Georges *et al*., 1995 ; Zhang *et al*., 1998 ; Heyen *et al*., 1999 ; Schrooten *et al*., 2000). To date, there has been little consensus on how data from QTL studies should be analyzed and what significance thresholds should be used to detect and report QTL. Therefore, it is not surprising that some of the QTL detected in these studies have been similar, while others have been unique to a specific experiment.

Several studies (Zhang *et al*., 1998 ; Kühn *et al*., 1999 ; Wiener *et al*., 2000 ; Nadesalingam *et al*., 2001) identified QTL affecting milk composition traits on BTA6. None of these studies have determined the total number of QTL on the chromosome nor fine-mapped the location of the QTL. In our genome scan, we detected a highly significant QTL on BTA6 affecting milk protein and fat percents in one U.S. Holstein family using the granddaughter design. Due to the large number of statistical tests involved in the genome-scan and the simplistic nature of the analysis, the significant marker-trait association must be studied further to refine the QTL map position.

MATERIALS AND METHODS

Source of Materials. DNA extraction and PCR amplification protocols were previously described (Ashwell *et al*., 1996 ; Ashwell *et al*., 1997). DNA samples from one family used in our genome scan (Ashwell *et al*., 2001) were again studied to refine the location of the putative QTL. A total of 91 sons were genotyped. Phenotypic data for milk yield and composition, somatic cell score (SCS), and productive life collected through November 2001 were processed as part of the genetic evaluation procedure by the Animal Improvement Programs Laboratory of USDA-ARS.

Fine Mapping. For QTL location refinement, 19 markers from the USDA map (http://www.marc.usda.gov) and 9 unpublished microsatellite markers (T.S. Sonstegard, unpublished) were selected for this study.

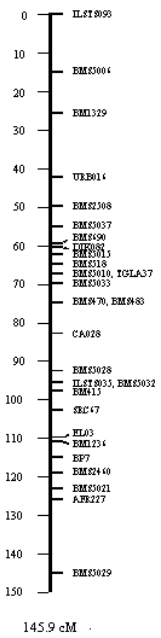
Construction of BTA6 linkage map. Marker order and relative map distances for the 28 microsatellite markers were estimated using CRI-MAP v. 2.4 (Green *et al*., 1990) from genotypic data generated in the families selected for our genome scan (Ashwell *et al*., 2001) and fine-mapping studies. Approximately 1600 bulls were genotyped and used in linkage map construction.

Statistical Analyses.

QTL Express. Interval analysis was conducted using the regression approach described by Haley and Knott (1992). The web-based version (Seaton *et al*., 2001), QTL Express, was used to analyze the data within the family. The software allowed the fitting of one and two QTL and included tools to calculate chromosome-wise significance thresholds and 95 % confidence intervals. The chromosome-wise significance thresholds were calculated using 1000 permutations. One thousand re-samples were selected for bootstrapping to determine the 95 % confidence intervals, and analysis was conducted at 1-cM intervals. Daughter deviations (DD) for milk yield, protein yield, protein percent, fat yield, fat percent, productive life and SCS traits were analyzed, weighted using their respective reliabilities.

SOLAR. Interval mapping was performed using the program SOLAR v1.7.3 (Almasy and Blangero, 1998) according to the documentation provided with the software. Twopoint analysis was performed for each marker using the TWOPOINT command. Multipoint identity by decent (MIBD) values were generated at 1cM intervals using the MIBD command and the male linkage map. Multipoint QTL interval analysis was conducted at 1cM intervals using the MULTIPOINT command.

RESULTS



BTA6 linkage map. A linkage map of chromosome 6 was constructed (figure 1) using genotypes generated from the Holstein families we selected for our QTL studies. Marker order is comparable to the USDA BTA6 order except for markers towards the telomeric end of the map. Markers *ILSTS035* and *BM415* are flipped and marker *BP7* was placed proximal to *BMS2460*. Marker order and relative positions determined from these families were used for the interval analysis.

QTL Express mapping results. In our genome scan (Ashwell *et al*., 2001), a simplistic single-marker approach was used analyze data to identify suggestive marker-QTL associations. Based on the highly significant ANOVA results for *BP7* and *BM415* from the scan, additional markers from the USDA BTA6 linkage map were genotyped in the family to begin fine-mapping of milk protein and fat percentage QTL on this chromosome.

Regression interval analysis was completed, first, fitting one QTL in the model, and then fitting 2 QTL. Results provided strong evidence (F statistic = 49.15, LOD = 8.43) of one QTL affecting protein percent near marker *BMS5037* (figure 2), with the 95 % CI from 56-61 cM. Results also showed evidence (F statistic = 21.77, LOD = 4.2) of one QTL affecting milk fat percent near the same marker. No significant effects on the remaining traits (modeling both one and two QTL) were observed (data not shown).

Figure 1. Male-specific linkage map of BTA6

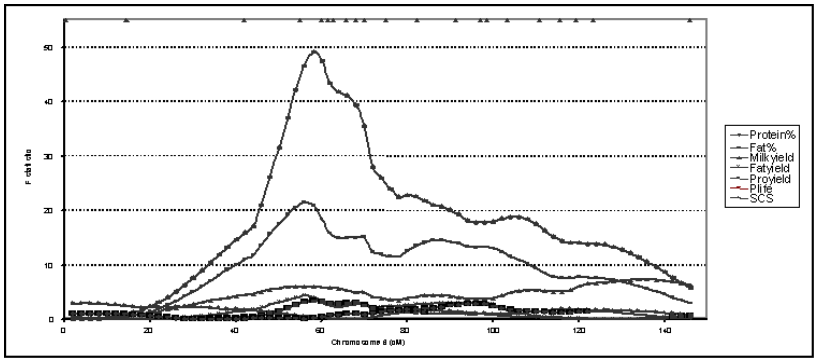


Figure 2. QTL Express interval mapping results, fitting one QTL in the model
Informative marker locations are shown as triangles at the top.

SOLAR mapping results. Variance components analysis was performed using SOLAR. Results provided strong evidence (LOD = 6.26) of a QTL affecting milk protein percent at 65cM, near marker *BMS518* (figure 3). There was suggestive evidence (LOD = 1.97) of a QTL affecting milk fat percent at 33cM. No significant effects on the remaining traits were observed.

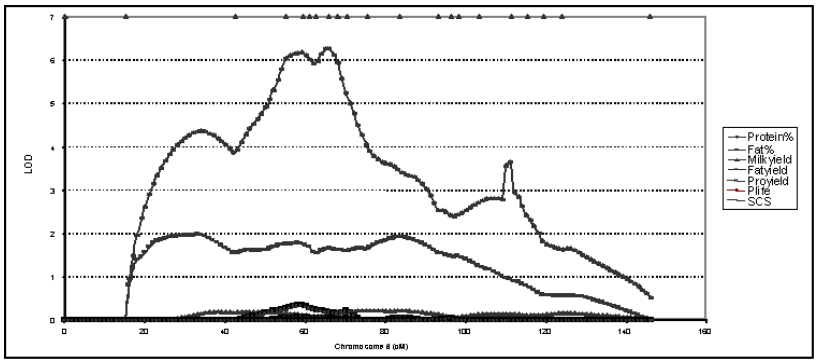


Figure 3. SOLAR interval mapping results
Informative marker locations are shown in triangles at the top.

DISCUSSION
A QTL affecting milk protein percent was identified using two different analysis methods. The placement of the QTL was comparable between the two analysis methods, placing the QTL between 55 and 65cM on the linkage map. The placement of this QTL is difficult to compare with results from other groups because the same markers were not genotyped across studies. However, the placement of the protein percent QTL described here is in general agreement with the QTL affecting fat and protein percent and milk yield described by Zhang *et al*. (1998). It is possible that the same family was studied by both groups, however, this cannot be

confirmed. We were not able to confirm a QTL affecting milk protein and fat yield as detected in German Holstein families (Kühn *et al*., 1999). Wiener *et al*. (2000) detected a QTL affecting milk, fat and protein yields near marker *BP7*. Although not detected by modeling two QTL in QTL Express, results obtained from SOLAR may suggest a second QTL on BTA6 affecting protein percent near *BP7* (figure 3).

CONCLUSION

Results from the study of one U.S. Holstein family provide strong evidence of a QTL affecting milk protein and fat percentage on bovine chromosome 6. Identification of markers that flank this QTL is expected to give rise to a greater rate of genetic improvement through incorporation of the marker information into the genetic evaluation system.

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