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Fine mapping quantitative trait loci affecting milk production traits on bovine chromosome 6 in a Chinese Holstein population

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Abstract

To fine map the previously detected quantitative trait loci (QTLs) affecting milk production traits on bovine chromosome 6 (BTA6), 15 microsatellite markers situated within an interval of 14.3 cM spanning from BMS690 to BM4528 were selected and 918 daughters of 8 sires were genotyped. Two mapping approaches, haplotype sharing based LD mapping and single marker regression mapping, were used to analyze the data. Both approaches revealed a quantitative trait locus (QTL) with significant effects on milk yield, fat yield and protein yield located in the segment flanked by markers BMS483 and MNB209, which spans a genetic distance of 0.6 cM and a physical distance of 1.5 Mb. In addition, the single marker regression mapping also revealed a QTL affecting fat percentage and protein percentage at marker DIK2291. Our fine mapping work will facilitate the cloning of candidate genes underlying the QTLs for milk production traits.

Keywords: fine mapping; QTL; *Bos Taurus autosome* 6; milk production traits

Introduction

Most economically important traits in farm animals are complex traits, which are controlled by a large number of polygenes with small effect and possibly a few genes with moderate or large effect on phenotypic variation. With the advances in molecular marker techniques, comprehensive research has been conducted to identify the chromosome regions harboring such genes, i.e., quantitative trait loci (QTLs), and considerable QTLs have been detected *via* genome scanning based on marker-QTL linkage analysis. However, the resolutions of these QTLs are usually poor and the confidence intervals are as large as 10–30 cM. With such large intervals it is hard to positionally clone the corresponding genes. Thus, the QTLs derived from genome scanning need to be narrowed to an interval within 1–2 cM.

In dairy cattle, chromosome 6 (BTA6) is one of the most concerned chromosomes in QTL mapping for milk production traits. Since the first report on QTL mapping in dairy cattle by Georges et al. (1995), 403 QTLs for milk production traits have been reported, 65 of which are on BTA6 (http://www.animalgenome.org/QTLdb/cattle.html, Oct. 20, 2008). In recent years, several fine mapping studies have been conducted for BTA6. Ron et al. (2001) fine mapped a QTL affecting protein and fat percentage to a 4 cM region around BM143 in an Israeli Holstein population. Olsen et al. (2004) fine mapped a QTL affecting fat percentage and protein percentage to a 7.5 cM interval surrounded by the markers BMS2508 and FBN12 (near BM143) in Norwegian Dairy cattle. This region was even further refined within a distance of 420 kb (Olsen et al., 2005). Schnabel et al. (2005) fine mapped a QTL affecting

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protein percentage to a small interval in the vicinity of BM143 in a U.S. Holstein population. These studies provide strong evidence for the existence of a QTL affecting fat or/and protein percentage located near BM143. Based on these fine mapping results, several strong candidate genes with potential effect on milk production traits were identified from these regions, including the *OPN* gene (Schnabel et al., 2005), the *ABCG2* gene (Cohen-Zinder et al., 2005), and the *PPARGC1A* gene (Weikard et al., 2005).

In our previous study in a Chinese Holstein population consisting of 26 sire families (Chen et al., 2006), we mapped a QTL affecting fat yield around BMS470, which is about 14cM away from BM143. Among the 26 families, we identified eight families in which one or more QTLs with significant effects on milk yield, fat yield, protein yield, fat percentage, or protein percentage are segregating. An analysis across the significant families revealed a significant QTL for milk yield, fat yield, protein yield and fat percentage at the same position (Chen et al., 2005). Through additional analysis across the significant families using the variance component approach (Chen et al., 2005), we mapped the QTL for milk yield and protein yield around BM470 with confidence intervals of 4 cM. This region was also reported to harbor one or more QTLs for milk production traits by several investigators (Zhang et al., 1998; Velmala et al., 1999; Szyda et al., 2005; Harder et al., 2006). The aim of the present study is to fine map this QTL by increasing the marker density in the region around BMS470 and employing linkage disequilibrium (LD) mapping approaches.

Materials and methods

Animals

All animals were from the eight sire families which had been proved to contain segregating QTLs affecting milk yield, fat yield, protein yield, or fat percentage in our previous study (Chen et al., 2006). The eight sires are unrelated (no known common ancestors). These families were enlarged with the total number of daughters increasing from 746 in the previous study to 918 in the current study. These animals were from 15 Holstein cattle farms in Beijing, China with an average 305-day milk yield of about 8,500 kg, where regular and standard performance testing (dairy herd improvement, DHI) has been carried out since 1999. The distribution of the animals in the eight families is given in Table 1.

Table 1
Sire families and number of daughters in each family

No.	Sire	No. of daughters
1	2085	101
2	2089	128
3	2090	93
4	2091	64
5	2102	233
6	2103	47
7	2105	51
8	2110	201
Total		918

Marker data

Based on the mapping results of our previous work (Chen et al., 2006), 14 evenly spaced microsatellite markers (Fig. 1) surrounding BMS470 on BTA6 were selected from the MARC2004 map (Ihara et al., 2004). They cover a 14.3 cM interval with an average distance of 1.1cM between adjacent markers. However, between markers BM4322 and BMS470 there was no markers available and the distance between them was 3.53 cM which is too long for fine mapping. To explore new markers in this interval, the DNA sequence of this region was downloaded from http://www.ensembl.org, and then subjected to SSRhunter3.0 (Li and Wan, 2005) for scanning new microsatellite markers. A new marker, named M1, located in the middle of this interval, was ultimately used in this study. The primers for this marker are 5′-CACA CACATACACCAACACAC-3′ (forward) and 5′-GTGC TTATATGCTTTCAGTCG-3′ (reverse). Its map position was interpolated between BM4322 and BMS470 based on its relative physical position (Christians and Keightley, 2004). The map positions, number of alleles, allele frequencies in the daughters, polymorphic information contents (*PIC*), and rates of completed genotyping of all markers used in the current study are given in Table 2.

DNA was extracted from blood of cows and semen of sires using the routine procedures. PCR of all markers was carried out on the GeneAmp PCR System 9600 or 9700 (PE, Applied Biosystems, Foster City, CA, USA) and followed by running the fluorescence labeled products on the ABI 377 DNA sequencer (Applied Biosystems, USA). The genotyping was done using GeneScan ver. 3.0 and Genotyper ver. 2.5 (Applied Biosystems).