A Significant Quantitative Trait Locus Affecting Protein Percentage On Bovine Chromosome 3 Was Detected In The Chinese Holstein

Qin Chu*†, Dongxiao Sun*, Yi Zhang*, Ying Yu*, Yachun Wang*, *Yuan Zhang**

Introduction

Most economically important traits of dairy, including production and functional traits, are all influenced by both polygenes and environment. In the past two decades, the development of genetic markers and statistical methods has made it possible to map and fine map of chromosome regions covering genes affecting economic traits. Many efforts have been undertaken in various cattle breeds all over the world. Data on QTL mapping increased rapidly. According to the Animal Quantitative Trait Locus (QTL) database (AnimalQTLdb) (http://www.animalgenome.org), until 2009, there have been 114 papers relating to QTL mapping, which reported 2 344 QTLs referring to 185 traits.

However, the genetic effect of any identified QTLs and linkage disequilibrium phase may differ across various populations because of specific background, so it's necessary to detect QTL in specific populations. In Chinese Holstein, previous researches were mainly focused on BTA6. Bovine chromosome 3 has been shown to harbor QTLs that influence milk production traits (Zhang, Q., Boichard, D., Hoeschele, I., et al. (1998); Heyen, D.W., Weller, J.I., Ron, M., et al. (1999); Ashwell, M.S., Van Tassell, C.P. and Sonstegard, T.S. (2001); Plante, Y., Gibson, J.P., Nadesalingam, J, et al. (2001); Olsen, H.G., Gomez-Raya, L., Våge, D.I., et al. (2002); Boichard, D., Grohs, C., Bourgeois, F., et al. (2003); Viitala. S.M., Schulman. N.F., de Koning. D.J., et al. (2003); Ashwell, M.S., Heyen, D.W., Sonstegard, T.S., et al. (2004)). The objective of this study was to search QTLs affecting milk production traits on BTA3 in the Chinese Holstein population, which is expected to facilitate the subsequent fine mapping and positional and functional gene cloning.

Material and methods

Animals. Fifteen half-sib families consisting of a total of 1 722 Chinese Holstein cows from 21 dairy herds in Beijing region were analyzed in a daughter design. The number of daughters per sire ranged from 91 to 229 with an average family size of 115 cows. The cow blood samples were collected from the local dairy herds in Beijing; the frozen semens of the 15 sires were provided by Beijing Dairy Cattle Center.

* Department of Animal Science and Technology, China agricultural university, Beijing, China
† Beijing Academy of Agriculture and Forestry Sciences, Beijing, China

Phenotypes. The 5 milk production traits, i.e. milk yield (MY), fat yield (FY), protein yield (PY), fat percentage (FP), and protein percentage (PP), were included in the analysis. Estimated breeding values (EBV) for the 5 traits of each cow were obtained from the Dairy Data Processing Center of China, which were calculated using the test-day model.

Genotyping. Seventeen microsatellite markers on BAT3 (*DIK4651, DIK2860, DIK4604, ILSTS096, BMS482, BL41, DIK4353, MB099, BMS937, DIK1165, MNS-21, DIK4833, BMS2145, BMS835, DIK2038, DIK2904,* and *DIK636*) were selected. These markers were evenly distributed on BTA3 with genetic distance between adjacent markers being from 5cM to 10cM.

The genomic DNA was extracted using the DNA DP318 kit (Tiangen, Beijing, China). PCR reactions were carried out using fluorescence-labeled primers. The PCR products were run on the ABI3700 DNA sequencer (Applied Biosystems Foster City, CA, USA) and the genotypes were determined by the ABI Prism GeneMapper 3.0 software.

Statistical Analysis. For comparison purpose, we chose the MARC2004 map (Ihara et al., 2004) as the linkage map. The interval mapping was performed for each trait separately across families using the online software GRIDQTL (Seaton et al., 2006; http://www.gridqtl.org.uk/index.htm). The model was,

$$EBV_{ij} = \mu + u_{ij} + \sum_{t}^{n} (v_{ijt}^{1} + v_{ijt}^{2}) + e_{ih}$$

where, μ was the mean, u_{ij} was the polygene effect of the j_{th} daughter from the ith sire, v_{ijt}^{k} was the k allele effect at the t_{th} QTL of the j_{th} daughter from the i_{th} sire, t was the number of QTL, equals 1 in this experiment, e_{ij} was the residual error.

To test the presence of a QTL, the likelihood ration statistic (LR) was calculated in 1 cM steps along the whole chromosome. Significance thresholds were determined by the Chi-square distribution with the degrees of freedom 1 to 2. The 95% confidence interval was estimated by the LOD drop-off method.

Results and discussion

The mapping results were shown in figure 1, in which the test statistic (likelihood ration, LR) for one QTL at a given location vs. no QTL was depicted across the whole chromosome. The highest peaks for traits MY, FY, PY, FP and PP appeared at 8cM, 51cM, 59cM, 33cM, and 52cM, respectively.

For higher statistical strictness, we chose the Chi-square distribution with the degree of freedom (df) 2 to determine the 5% significant threshold. However, only the maximum LR for protein percent was greater than the chi-square critical value (5.99). For this QTL, the 95% confidence interval was 25-57cM.

Boichard, D., Grohs, C., Bourgeois, F., et al. (2003) found a QTL for protein percentage at 24cM on BTA3, and the proportion of variance due to this QTL in the total genetic variance is only 7%. While in the present study, the proportion was 4.9%. Both indicated the minor effect of this QTL on protein percentage Therefore, more individuals and higher density map are needed to further investigate this region.

Several recent studies have reported the segregation of QTLs affecting milk production traits between markers *INRA006* (17.088cM) and *INRA003* (59.5cM) on BTA3 (Heyen, D.W., Weller, J.I., Ron, M., et al. (1999); Ashwell, M.S., Van Tassell, C.P. and Sonstegard, T.S. (2001); Plante, Y., Gibson, J.P., Nadesalingam, J, et al. (2001); Ashwell, M.S., Heyen, D.W., Sonstegard, T.S., et al. (2004)). In this study, the highest peak for trait PP appeared at 52cM and the 95% confidence interval was between 25cM and 57cM, which were inside such 17-59cM region appropriately.

When the Chi-square distribution with a single degree of freedom was used, there were two additional peaks higher than the 5% significant threshold ($\chi^2_{0.05}(1)=3.84$), one for MY at 8cM and the other for PY at 59cM. The QTL location of PY was similar to that reported by Ashwell , M.S., Heyen, D.W., Sonstegard, T.S., et al. (2004). A second peak for FY was observed near 59cM though it was not significant. The 95% confidence intervals of QTL for MY comprised two separated regions, 0-16cM and 49-67cM. The first one was most likely consistent with that mapped in the U.S. Holstein (Zhang, Q., Boichard, D., Hoeschele, I., et al. (1998); Heyen, D.W., Weller, J.I., Ron, M., et al. (1999)) and the Swiss brown population (Bagnato, A., Schiavini, F., Rossoni, A., Maltecca, C., et al. (2008)), while the second one lay within the previous reported region 56 ± 8.6cM (Khatkar. M.S., Thomson. P.C., Tammen. I., et al. (2004)).

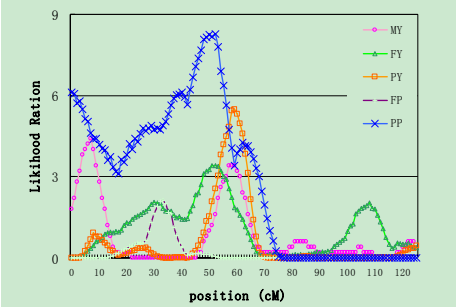

Figure 1: Likelihood ration for QTL affects on five milk production traits along BTA3

Conclusion

This study suggested the existence of a possible significant QTL affecting protein percentage in the region 25-57cM on BTA3 in the Chinese Holstein. This finding may facilitate fine mapping of QTL and the cloning of candidate genes on BTA3.

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