Search For QTLs On BTA6 Affecting Milk Production Traits In Chinese Holstein With SNP Markers

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Introduction

QTLs affecting milk production traits had been scanned on all 29 bovine autosomes. QTL mapping on bovine chromosome 6(BTA6) drew lots of attention because many independent studies in different populations detected QTL for milk production traits on BTA6 (Ashwell, M.S., Schnabel, R.D., Sonstegard, T.S. *et al.* (2002); Spelman R.J., Coppieters, W., Karim, L. *et al.* (1996); Freyer, G., Kühn, C., Weikard, R. *et al.* (2002); Wiener, P., Maclean, I., Williams, J.L. *et al.* (2000); Olsen, H.G., Lien, S., Svendsen, M. *et al.* (2004)).In this study, we used the newly submitted cow genome sequence map (http://www.enseml.org/Bos_taurus/Info/Index), genetic map(Ihara, N., Takasuga, A. Mizoshita, K. *et al*. (2004)) and physical map (Schibler, L. Roig, A., MahéM, F. *et al.* (2004); Van der Wind, A.E., Kata, S.R., Band, M.R. *et al.* (2004); Weikard, R., Goldammer, T. Laurent, P. *et al.* (2006)) to chose a region of 25 Mb in most concerned part of BTA6 and tried to use SNP markers to fine map milk production QTLs in Chinese Holstein by daughter design.

Material and methods

Animals and Traits: A total of 1440 Chinese Holstein dairy cows with a daughter design were tested which belongs to 11 sire families. The pedigree of each animal was traced back 3 generations, complex family relationships existed between the animals, and several of the sires were paternal half-sibs. Estimated breeding value (EBV) of all animals was obtained from Dairy Data Processing Center of China and was used as performance information in the analyses. Traits analyzed were milk yield (MY), fat yield (FY), protein yield (PY), fat percentage (FP), and protein percentage (PP).

Marker Map: The microsatellite map (*BMS2508-ILSTS097*) was increased by 15 SNPs which were generated by amplifying and sequencing genes likely to be positioned to the region on the basis of comparative mapping information (figure 1). We used physical distance on gene map to estimate the genetic distance for SNP markers (1cM≈1000kbp), and the map used in LOKI analysis was shown in table1. Sequencing was done on 11 sires

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using an ABI3730 sequencer. SNP detection was carried out by Aligner- software constructed on the basis of Phred, phrap, and polyphred programs. Marker order and map distances were estimated based on the newly bovine sequence map released on ensemble web site. The marker map of this study was shown in figure1.

Genotyping: SNP genotyping pipeline was constructed on basis of molecular character of the SNP site. RFLP, AFLP, and TaqMan probe methods were used.

Linkage analysis: LOKI was used for multipoint QTL analysis across-family and within family to estimate both the number and positions of QTLs. The test statistics Bayes Factor (BF) is the average over iterations of posterior/prior ratio (*q/p*) for each linkage group. The signal criterion is: 3≤BF≤20 is considered as positive signal, 20≤BF≤150 as strong signal,150≤BF as very strong signal.

Before running the LOKI program, the parameter files and data files were prepared. The data files consist of pedigree, phenotype (EBV) and genotype, the parameter files consist of the number of iteration, marker map location, random seedfile and the character of the loci affected traits etc. We set iteration number as 100,000 and the position of SNPs markers were set as Tabe 4 which refer to the microsatellite position on RH map and genetic map. The character of the loci affected traits declare as quantity trait loci, and the number of QTL set 0-5. We use the seedfile included in the package as seed.

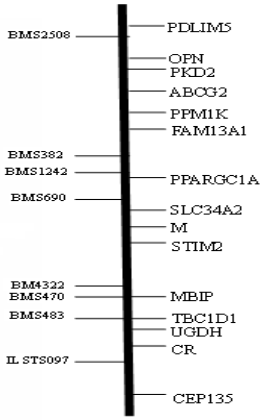

Figure 1 Sketch map of SNP markers in target region

Table1: Map Position of each Marker with LOKI(cM)

PDLIM5	*OPN*	*PKD2*	*ABCG2*	*PPM1K*	*FAM13A1*	*PPARGC1A*	*SLC34A2*
0.0	2.0	2.1	2.2	3.4	6.2	8.0	9.8
M	STIM2	MBIP	TBC1D1	UGDH	CR	CEP135	
10.2	11.7	14.7	16.5	17.7	18.1	25.6	

Results and discussion

A QTL affecting MY was detected between markers *PPARGC1A* and *SLC34A2* (BF=5.26) in across family analysis whereas there was no positive signal for QTL of FY, PY, FP, PP. Weikard, R., Kuhn, C., Goldammer, T. *et al* (2005) charactered and sequenced the *PPARGC1A* gene and found association of SNP with milk production traits. Khatib, H., Zaitoun, H., and Wiebelhaus-Finger, J. *et al* (2007) found SNP of *PPARGC1A* had significant additive effects for decreasing milk yield in UW resource population. So *PPARGC1A* could be a convictive physical candidate gene for milk yield.

Within-family analysis result: Two closely located QTLs affecting MY were detected in S4 in region CR-CEP135(BF=3.71, BF=7.00)and there was another positive signal beside *OPN* marker in S3 family analysis(BF=3.25). For PY QTL we found a single positive signal in S9 and S2 family which was in the region *PPARGC1A-SLC34A29*(BF=3.89) and *TBC1D1-UGDH* (BF=3.02）respectively. One QTL affecting FY was detected in region *UGDH-CR* (BF=4.34) in S4 family analysis. However, we did not find QTL segregation for FP and PP.

The linkage analysis within family results tell us that QTLs affecting the same trait were mapped in different region on chromosome in different family, they maybe one and the same QTL, or they were quietly different QTL, because QTL alleles could segregate differently in different family with different selection intensity or genetic draft.

There was no conclusion about which population configuration can get higher reliable result. Generally speaking, marker information content changed largely in within-family analysis, for allele frequency of one site change will cause change of test statistics and produce maximum.

For daughter design, result of within-family analysis could get further confirmation if the population is larger enough. If we found QTL segregation in one family, we could look for physical candidate gene in the QTL located chromosome region and carry out the candidate gene analysis.

Conclusion

We detected QTL segregation in the Chinese Holstein population with daughter design, and QTL affecting milk yield beside *PPARGC1A* detected in across family analysis had been verified by linkage analysis within family, which is consistent with previous report. *PPARGC1A* could be a convictive physical candidate gene for milk yield.

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