

Linkage Mapping and QTL on Chromosome 6 in Hanwoo (Korean Cattle)

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ABSTRACT : The QTL(quantitative traits loci) linkage mapping of Hanwoo (Korean Cattle) chromosome 6 for daily gain and marbling score was performed using 378 individuals from 18 paternal half-sib families in Hanwoo. Hanwoo chromosome 6 were mapped to total length of 394.2 cM between 28 microsatellite loci using 36 microsatellite primers of BTA 6 linkage group. The QTL analysis for daily gain in Hanwoo showed 8 microsatellite loci (BM3026-5.66, EL03-5.58, BM4311-5.29, ILSTS035-4.50, BMS1242-4.37, BM1329-3.67, BM415-3.11, BMS2460-3.03) in larger than LOD score 3.0. Based on the QTL analysis for marbling score, LOD scores of 12 microsatellite loci (BM415-8.88, BM3026-7.15, ILSTS093-5.45, ILSTS035-4.91, EL03-4.69, BMS690-4.52, BM1329-4.43, BMS511-3.74, BMS1242-3.66, BMS518-3.65, BM4311-3.41, BMC4203-3.36) were found larger than 3.0. (*Asian-Aust. J. Anim. Sci.* 2003. Vol 16, No. 10 : 1402-1405)

Key Words : Hanwoo (Korean Cattle), Linkage Mapping, QTL, Marbling Score, Daily Gain, Microsatellite

INTRODUCTION

Most of the economic traits in cattle are dependent on polygene mechanism and thus any single gene effect has not been clearly reported. The progress in latest DNA technology was applied to improve these traits as genetic linkage maps providing the foundation for mapping quantitative trait loci (QTL) associated with growth rate, meat quality, carcass, and reproduction trait in cattle.

In cattle, genetic linkage mapping has been reported in all chromosomes (Bishop et al., 1994; Georges et al., 1995; Barendse et al., 1997; Kim et al., 2000). Studies for economic traits were published on meat tenderness in steers (Keele et al., 2000; Rexroad et al., 2001), a QTL affecting carcass and growth traits (Taylor et al., 1999; Stone et al., 1999; Casas et al., 2001), and meat quality in Wagyu (Hirano et al., 1998). Recently, Casas et al. (2000) has reported that a QTL for birth and yearling weight segregated on chromosome 6 from Belgian Blue inheritance (n=246) by seven microsatellite loci (INRA133, BM1329, BMS2508, BMS382, BM3026, BMS483 and BM415).

Hanwoo (Korean cattle) as a native genetic source in Korea has entered upon a new phase in opening beef market because of retarded growth and expensive production cost. On the other hand, beef quality based on marbling score is the important competitive factor to other imported beef. Therefore, here we report the linkage map of Hanwoo based on the USDA-MARC BTA 6 linkage group and QTL analysis for daily gain and marbling score.

MATERIALS AND METHODS

Animals and trait

Three hundred seventy eight steers from 18 paternal half-sib families were used for linkage mapping and QTL from Hanwoo Improvement Center, National Agricultural Cooperation Federation, Korea. Daily gain from birth to 720 days of age and marbling scores at slaughter of 720 days of age were measured. Marbling score was scored as 19 degrees and classified by 1+, 1, 2 and 3 for market systems. The grading of marbling score was measured according to standards of the Korean Animal Products Grading Service.

Genomic DNA isolation and microsatellite analysis

Genomic DNA from white blood cells was extracted by the phenol-chloroform method (Sambrook and Russell, 2001). Thirty-six microsatellite primers (ILSTS093, BMS3, INRA133, ILSTS090, BM1329, BMS382, BMS1242, BM3026, BMS690, BMS518, BM4322, BMS470, BMS483, BMS360, ILSTS097, BM4528, BM4621, CA028, BM415, ILSTS035, EL03, ILSTS087, CSPS100, BM1236, BMS2460, BM4311, ILSTS018, BMS511, AFR227, BP7, BM8124, ETH8, JMP8, BMC4203, BM2320 and BL1038) for PCR amplification were designed from the USDA Meat Animal Research Center BTA 6 linkage group (<http://sol.marc.usda.gov/marc/html/gene1.html>).

Polymerase chain reaction (PCR) for microsatellite was performed using 20 ng of Hanwoo genomic DNA as template with 1×PCR buffer, 1 mM MgCl₂, 0.2 mM dNTPs, 15 pM of each primer, and 1 unit of *Taq* polymerase (Takara Co., Japan) in 30 µl reaction volume. The PCR was performed for 5 min at 94°C, and followed 35 cycles of 30 sec at 94°C, 30 sec at 50-60°C, 1 min at 72°C, and concluding with a final extension of 10 min at 72°C. The

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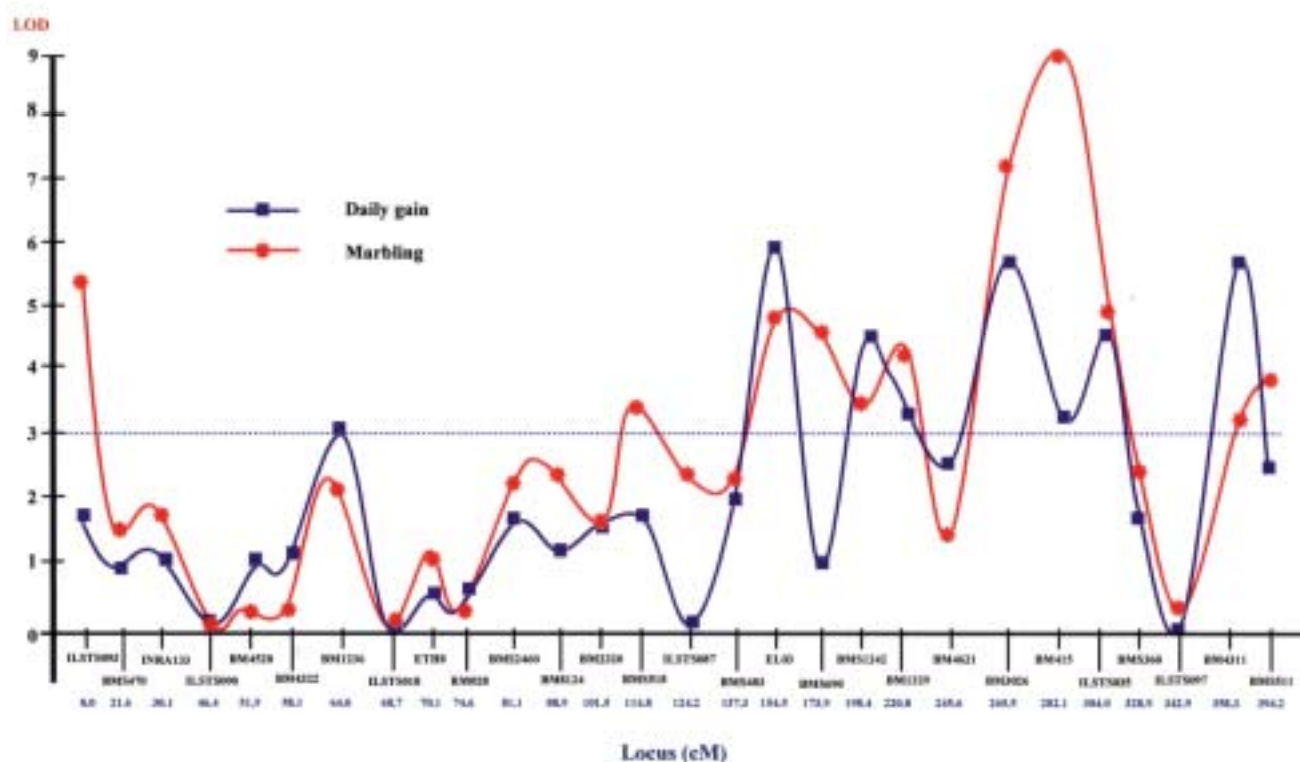


Figure 1. LOD scores for daily gain and marbling score on Hanwoo (Korean Cattle) chromosome 6 linkage map.

PCR products were analyzed in 6% polyacrylamide gel electrophoresis. The exact microsatellite alleles were determined with 10 and 100 bp standard markers.

Linkage and QTL analysis

Two-point linkage analyses were performed with the software package CRI-MAP (Green et al., 1990). Linkage map of chromosome 6 in Hanwoo were derived from preliminary two-point and multi-point analyses with the BUILD and Flips options of CRI-MAP. Two-point linkage analysis was used to LOD score greater than 3.0 with linkage between markers.

A linear and maximum likelihood method (Lander and Botstein, 1989) for QTL analysis was used.

RESULTS AND DISCUSSION

Linkage map

We found that only 28 microsatellite loci localized on the chromosome 6 in Hanwoo by linkage analysis using 36 microsatellite primers of USDA-MARC BTA 6. The total length of the chromosome 6 in Hanwoo was 394.2 cM (Figure 1). In the total length of 394.2 cM, the linkage distances between 1.4 cM and 20 cM were group 74%. The distances of the linkage groups between 20 cM and 35.9 cM were 26%. The BTA 6 genetic map reported that total linkage interval was 125.6 cM with 46 microsatellite loci.

This result has been shown different linkage distance between BTA 6 and Hanwoo chromosome 6. An average linkage interval of BTA 6 was 2.7 cM. However, Hanwoo chromosome 6 linkage interval was 11.8 cM (Figure 1). This discrepancy between BTA 6 and Hanwoo 6 might be caused by difference in reference population. Selection of reference families for this linkage study had been limited in using artificially selected families and low genetic variation from government breeding system of progeny test for above 20 years. Although reference population of Hanwoo in this study was half-sib families of progeny test known lower genetic homogeneity compared to other breeds (Yeo et al., 1997), recombinant (crossing-over) values between linkage loci could not appear as same result of USDA-MARC BTA 6. In order to make more precise Hanwoo linkage mapping using half-sib population, large numbers of individuals and progenies per family, and distinctive genetic markers rather than full-sib reference population (Lander and Botstein, 1989) are considered to be necessary.

QTL detection for daily gain and marbling

The QTL mappings of daily gain and carcass traits in cattle (Stone et al., 1999; Casas et al., 2000, 2001; Li et al., 2002) have been reported. Through the implied reports that QTL related to growth and carcass traits were located chiefly in chromosomes 6 and 27 (Casas et al., 2000), the QTL analysis for daily gain and marbling score based on

Table 1. The LOD score for daily gain and marbling score based on Hanwoo chromosome 6 linkage group

Loci	LOD score	
	Daily gain	Marbling score
ILSTS093	1.69	5.45
BMS470	0.93	1.57
INRA133	1.21	1.82
ILSTS090	0.14	0.07
BM4528	0.99	0.43
BM4322	0.82	0.20
BM1236	3.03	2.24
ILSTS018	0.01	0.10
ETH8	0.45	0.98
RM028	0.28	0.38
BMS2460	1.49	2.37
BM8124	1.19	2.65
BM2320	1.30	1.39
BMS518	1.37	3.65
ILSTS087	0.19	2.31
BMS483	1.81	2.16
EL03	5.58	4.69
BMS690	0.82	4.52
BMS1242	4.37	3.66
BM1329	3.67	4.43
BM4621	2.59	1.37
BM3026	5.66	7.15
BM415	3.11	8.88
ILSTS035	4.50	4.91
BMS360	1.73	2.21
ILSTS097	0.13	0.61
BM4311	5.29	3.41
BMS511	2.22	3.74

chromosome 6 in Hanwoo are shown in Figure 1. The LOD score range to daily gain was between 0.07 and 8.88 (Table 1). Eight loci were above 3.0 LOD score (BM3026-5.66, EL03-5.58, BM4311-5.29, ILSTS035-4.50, BMS1242-4.37, BM1329-3.67, BM415-3.11, BMS2460-3.03). Marbling score ranged 0.01 to 5.66 LOD score and 12 loci (BM415-8.88, BM3026-7.15, ILSTS093-5.45, ILSTS035-4.91, EL03-4.69, BMS690-4.52, BM1329-4.43, BMS511-3.74, BMS1242-3.66, BMS518-3.65, BM4311-3.41, BMC4203-3.36) were calculated more than 3.0 LOD score.

Although several loci of chromosome 6 in Hanwoo were related with daily gain and marbling score, LOD scores of microsatellite loci related with daily gain were found higher than marbling score. Therefore it was considered that microsatellite loci of chromosome 6 in Hanwoo are strongly related with daily gain. Actually microsatellite locus above 3.0 LOD score has difficulties to use as a selection marker (MAS) because each loci was not appeared simple allele in paternal half-sib linkage mapping differently full-sib. As next step to apply those loci, several alleles within each locus should be classified again with performances.

This report is the first linkage analysis and QTL

detection in Hanwoo. However, because we could use the paternal half-sib of the Hanwoo population and the small size of reference families, Hanwoo linkage group in this study showed large intervals between microsatellite loci. Further studies for Hanwoo genetic analysis should be done with all 30 chromosomes in Hanwoo and also marker assisted-selection (MAS) based on QTL analysis should continuously be studied. Hanwoo linkage map and QTL data demonstrated in the current study might be useful tools for the improvement system of genetic performance and the individual selection in Hanwoo breeding.

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REFERENCES

- Barendse, W., D. Vaiman, S. Kemp, Y. Sugimoto, et al. 1997. A medium density genetic linkage map of the bovine genome. *Mamm. Genome* 8:21-28.
- Casas, E., S. D. Shackelford, J. W. Keele, R. T. Stone, S. M. Kappes and M. Koohmaraie. 2000. Quantitative trait loci affecting growth and carcass composition of cattle segregation alternate forms of myostatin. *J. Anim. Sci.* 78:560-569.
- Casas, E., R. T. Stone, J. W. Keele, S. D. Shackelford, S. M. Kappes and M. Koohmaraie. 2001. A comprehensive search for quantitative traits loci affecting growth and carcass composition of cattle segregating alternative forms of the myostatin gene. *J. Anim. Sci.* 79:854-860.
- Georges, M., D. Nielsen, M. Mackinnon, A. Mishra, et al. 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. *Genetics* 139:907-920.
- Green, P., K. Falls and S. Crooks. 1990. Documentation for CRIMAP, version 2.4. Washington University School of Medicine, St. Louis, MO.
- Hirano, T., N. Kobayashi, T. Nakamaru, K. Hara and Y. Sugimoto. 1998. Linkage analysis of meat quality in Wagyu. 16th International Conference on Animal Genetics, Auckland, New Zealand: E019.
- Keele, J. W., S. D. Shackelford, S. M. Kappes, M. Koohmaraie and R. T. Stone. 1999. A region on bovine chromosome 15 influences beef longissimus tenderness in steers. *J. Anim. Sci.* 77:1364-1371.
- Kim, J. W., T. K. Jang, Y. A. Park and J. S. Yeo. 2000. Linkage mapping of chromosome 6 in the Korean Cattle (Hanwoo). *Asian-Aust. J. Anim. Sci.* 13 (Suppl.):235-235.
- Lander, E. S. and D. Botstein. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185-199.
- Li, C., J. Basarab, W. M. Snelling, B. Benkel, B. Murdoch and S. S. Moore. 2002. The identification of common haplotypes on bovine chromosome 5 within commercial line of *Bos taurus* and their associations with growth traits. *J. Anim. Sci.*

- 80:1187-1194.
- Rexroad, C. E., G. L. Bennett, R. T. Stone, J. W. Keele, S. C. Fahrenkrug, B. A. Freking, S. M. Kappes and T. P. Smith. 2001. Comparative mapping of BTA15 and HSA11 including a region containing a QTL for meat tenderness. *Mamm. Genome* 12:561-565.
- Sambrook, J. and D. W. Russell. 2001. *Molecular cloning*. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY. pp. 6.28-6.30.
- Stone, R. T., J. W. Keele, S. D. Shackelford, S. M. Kappes and M. Koohmaraie. 1999. A primary screen of the bovine genome for quantitative trait loci affecting carcass and growth traits. *J. Anim. Sci.* 77:1379-1384.
- Taylor, J. F., S. K. Davis, J. O. Sanders, J. W. Turner, J. W. Turner, R. K. Miller and S. B. Smith. 1999. Identification of QTLs for growth and carcass quality in a cross between *Bos indicus* and *Bos taurus*. Plant-Animal Genome Conference, San Diego, CA: W19.
- USDA-MARC. Bovine Gene Map. <http://sol.marc.usda.gov/genome/cattle/cattle.html>.
- Yeo, J. S., J. W. Kim, E. J. Lee, M. Y. Lee and Y. H. Yang. 1997. Genetic analysis of cattle breeds using DNA fingerprinting. *Korean J. Anim. Sci.* 39:641-646.