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Detection of Quantitative Trait Loci for Growth and Carcass Traits on BTA6 in a Hanwoo Population

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ABSTRACT : The purpose of this study was to detect quantitative trait loci (QTL) for growth and carcass quality traits on BTA6 in a population of Hanwoo cattle. Three hundred and sixty one steers were produced from 39 sires that were sired by 17 grandsires in the two Hanwoo farming branches of the National Livestock Research Institute of Korea, between Spring 2000 and Fall 2002. DNA samples were collected for all of the steers, sires and grandsires, and the phenotypes for six growth and carcass quality traits were measured at 24 months of age. Twelve microsatellite markers were chosen on BTA6 and a linkage map was constructed by using seven of the twelve markers. Then, a chromosome-wide QTL scan was performed by applying an Animal Model, in which effects of QTL alleles within the grand sires were fitted as a random term. Three QTL were detected at the 5% chromosome-wise level for backfat thickness, average daily gain, and final weight. The most likely positions for the QTL were in the proximal region, i.e. 0 cM, 35 cM, and 63 cM, respectively. Also, another QTL for *longissimus dorsi* muscle area was detected at the 10% chromosome-wise level at 67 cM. These results were, in general, consistent with our previous report, in which candidate gene analyses showed that a SNP near ILSTS035 flanked by BM4621 (62.5 cM) and BMS2460 (81.3 cM) was associated with final weight, carcass weight, average daily gain, and *longissimus dorsi* muscle area in the same Hanwoo population. (**Key Words :** QTL, BTA6, Growth, Carcass Traits, Hanwoo)

INTRODUCTION

Studies on the detection of quantitative locus or candidate genes that are associated with economically important traits in beef cattle have been extensively performed and many QTL or SNPs of candidate genes for their respective traits were reported (www.animalgenome.org). QTL studies that mainly focused on initial genome scans require structured pedigrees such as experimental F_2 or commercial paternal halfsib populations (Kim and Park, 2001). However, most of the QTL detection in livestock production was reported in developed countries, because implementation of QTL research requires well kept pedigrees going back at least several generations as well as large sample size requiring significant experimental costs. Instead, an alternative option is the candidate gene approach, in which linkage disequilibrium between loci of the tested candidate gene polymorphism and the causal gene mutations is exploited. This approach does not necessarily require structured pedigrees, linkage maps, and thus can be applied without estimation of the candidate gene position. Therefore, this approach can be applied to any type of (unstructured) population, and the verified SNP markers can be directly used in marker-assisted selection (MAS) schemes (Kim and Park, 2001). However, one of the considerable drawbacks of the candidate gene studies is that searching for QTL is confined, *a priori*, to the physiological categories of candidate genes (Rothschild and Soller, 1997).

Hanwoo, the Korean aboriginal breed, has been domesticated and evolved on the Korean peninsula, so as to have unique genetic characteristics (Decker et al., 2009). The Hanwoo beef has also become the favored choice of Korean meat consumers due to its high meat quality (Cho and Ko, 1998). QTL studies in Hanwoo cattle have been extensively performed in the last couple of decades (Kim et al., 2004; Cheong et al., 2007; Lee et al., 2008). However, all of the reports were based on the candidate gene approach, due to the limitation in implementing interval mapping methods for whole genome scan, as described above.

In this study, we first report QTL for carcass quality

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traits that were detected on bovine chromosome (BTA) 6 in Hanwoo cattle, by using linkage maps and an interval mapping method in paternal halfsib families.

MATERIALS AND METHODS

Animals and phenotype and molecular data

The Hanwoo data comprised of paternal grandsire halfsib pedigrees, with 361 steers produced from 39 sires that were sired by 17 grandsires. The number of steers per paternal grand-sire halfsib family ranged from 7 to 48, with an average of 21.2. The steers were born between Spring 2000 and Fall 2002, and they were raised in two different regions, Daekwanryeong and Namwon, of the National Livestock Research Institute in Korea, under the progeny testing program directed by the Korean Animal Improvement Association (Seoul, Korea). After 6 months of age, they were fed with concentrates consisting of 15% crude protein (CP)/71% totally digestible nutrients (TDN) during 60 to 90 days of age; 13% CP/72% TDN during 90 to 120 days of age as a self-feeding. Roughage was offered *ad libitum*.

All steers were slaughtered at an approximately 24 months of age. Carcasses were dissected at the last rib and the first lumber vertebra according to the Animal Product Grading System of Korea to measure carcass quality traits. Traits measured were final weight before slaughter (WT), carcass weight after slaughter (CWT), backfat thickness (BFT), *longissimus dorsi* muscle area (LMA), marbling score (Marb), and average daily gain (ADG) between 6 (weaning) and 24 months of age. The Marb score was numbered from 1 to 19 according to the Korean Beef Marbling Standard (1 = trace, 19 = very abundant). Table 1 shows the summary statistics for the observed growth and carcass quality traits.

Samples of DNA from 361 steers, 39 sires, and 17 grandsires were prepared from blood according to standard protocols, and the DNA concentration was adjusted to 20 ng/μl. Of the microsatellite markers on BTA6 listed in database (http://sol.marc.usda.gov/cattle, Kappes et al., 1997), twelve markers were chosen. Polymerase chain reaction was performed in a volume of 15μl containing 20 ng of genomic DNA, 1.25 m*M* of $MgCl_2$, 5 pmol each primer, 0.2 m*M* of deoxynucleotides (dNTPs), and 0.5 U of *Taq* DNA polymerase (SolGent Co., Ltd. Korea). The thermal cycling conditions were optimized for each primer set as in Kappes et al. (1997), and the other reaction conditions were set as recommended by the manufacturer. Following polymerase chain reaction, alleles were resolved by the Genetic Analyzer 3130XL instrument (Applied Biosystems, Foster City, CA) and genotype data were collected using GeneMapper v4.0 software (Applied Biosystems, Foster City, CA). Linkage maps were constructed using Cri-Map version 2.4 (Green et al., 1990). Build and Flip options were used to get the best marker order. Among the twelve microsatellite markers, only seven markers had significant linkage (LOD<3.0). The linked markers and their positions (Kosambi cM) were IL90 (0), BMS2508 (14.5), BMS518 (34.3), BM4621 (62.5), BMS2460 (81.3), BM8124 (117.4), and BMC4203 (175.3), respectively.

QTL analysis model

An extension of Animal Model, in which QTL alleles were fitted as random, was applied (Grignola et al., 1996a). The model is based on modeling covariances among relatives at individual marked QTL and assigning random effects to the QTL alleles within the grand sires. This model incorporates variance components due to the polygenic and QTL allele effects, and is

$$Y = X\beta + Zu + ZTv + e$$

Where Y is a vector of phenotypes, X is a design matrix, β is a vector of fixed and covariate effects, Z is an incidence matrix relating records to individuals, u is a vector of residual additive (polygenic) effects, T is an incidence matrix relating n individuals to 2n alleles at each QTL, v is a vector of allelic effects of the QTL, and e is a vector of uncorrelated residuals with constant variance. Fixed effects were included for the year-season of birth and birth-place, and a covariate for slaughter age was also fitted in the model. Pedigree information, i.e. between steers and grandsires, was used to specify Var (*u*) and Var (*v*). Four

Table 1. Summary statistics of observations on growth and carcass quality traits for 361 Hanwoo steers

Trait	Average	Std Dev[a]	Minimum	Maximum	C.V.[b]
Average daily gain (kg/d)	0.75	0.10	0.44	1.00	12.8
Final weight (kg)	567	65.4	328	741	11.5
Carcass weight (kg)	316	35.0	187	415	11.1
Backfat thickness (mm)	7.66	3.18	2	21	41.5
Longissimus dorsi muscle area (cm^2)	74.9	7.90	30	103	10.5
Marbling score (1-19)	4.58	3.50	1	19	76.3

[a] Standard deviation. [b] Coefficient of variation (%).

unknown parameters were fitted in the model: heritability (h^2), the fraction of additive genetic variance explained by QTL allelic variance (v^2), residual variance (σ_e^2), and QTL map position (p). The REML solution is obtained at the maximum of the restricted likelihood at which point a likelihood-ratio test statistic (LRT) of the form -2 $\ln(L_0/L_1)$ can be constructed to test hypotheses concerning v^2. A test for the presence of a QTL is constructed with L_0 the maximum value of the likelihood under the null hypothesis of no QTL allelic variance ($\sigma_v^2 = 0$), and L_1 the maximum value of the likelihood under the alternative hypothesis ($\sigma_v^2 > 0$). For the significance threshold, 5% chromosome-wise P value (LRT value of 3.84) was obtained from chi-square distribution with one degree of freedom. This value was based on the simulation results from Kim et al. (2003), in which under the simulation of no QTL in test chromosomes of length larger than 100 cM with low marker density (>10 cM of intermarker interval size), the empirical chi-squared distributions for the LRT statistics under the condition of no QTL was close to 1 degree of freedom.

RESULTS AND DISCUSSION

The BTA6 sex-average linkage map comprising seven markers spanned 175.3 K cM, whose length was larger than the distances in USDA MARC bovine genome map (http://www.animalgenome.org). However, marker orders were the same, and the relative distances between the markers ranging from 0 cM to 81 cM were not much different from the UDSA-MARC map, in which all of the QTL were detected in this study, i.e. for BFT, WT and ADG (Figure 1). The distal region of BTA6, however, was much larger compared to the MARC linkage map. This may be partly due to the fact that the number of steers per sire was not large enough to produce appropriate recombinants in the region.

Three QTL were detected on BTA6 at the 5% chromosome-wise level for BFT, ADG, and WT. The most likely positions for the QTL were in the proximal regions; 0 cM, 35 cM, and 63 cM, and their relative positions were close to the markers, IL90, BMS518, and BM4621, respectively (Table 2 and Figure 1). The proportions of

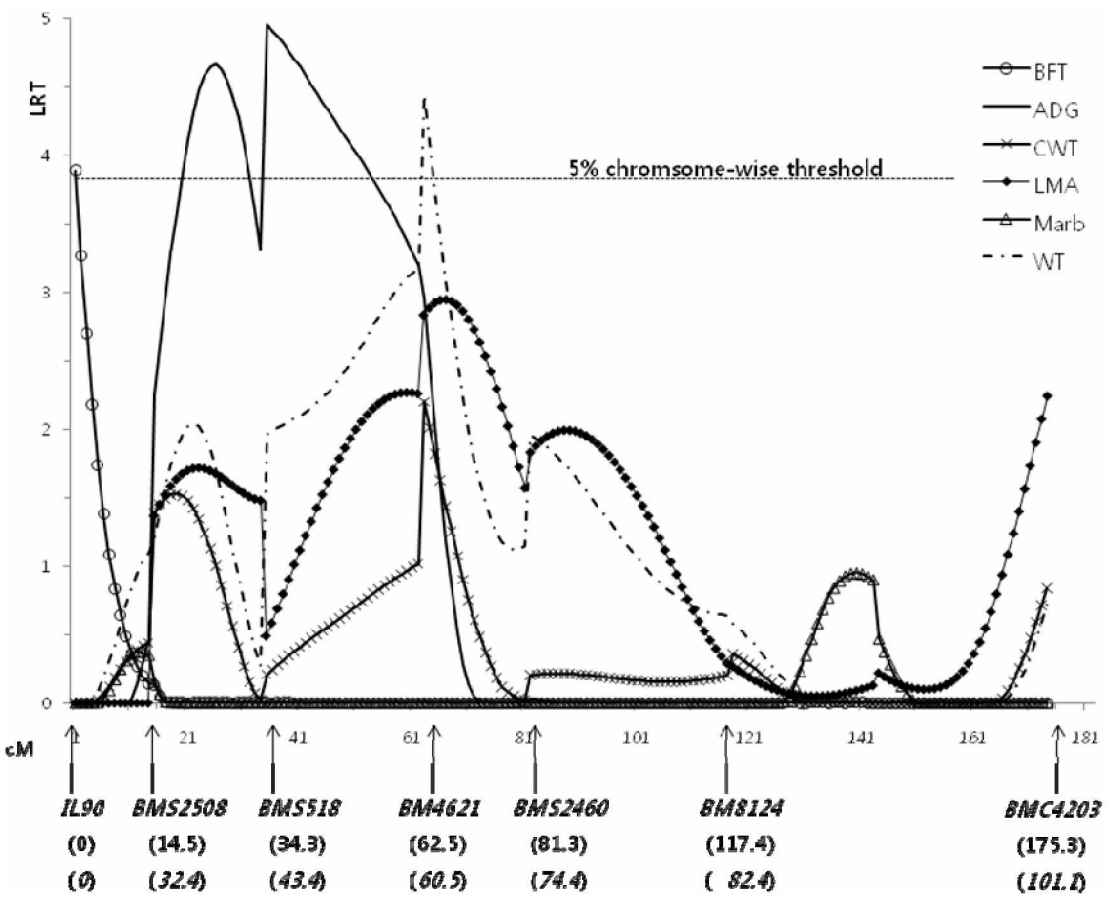

Figure 1. Likelihood ratio test statistic (LRT) profiles for growth and carcass quality traits on BTA6 (BFT = Backfat thickness; ADG = Average daily gain; CWT = Carcass weight; LMA = *Longissimus dorsi* muscle area; Marb = Marbling score; WT = Final weight). The upper and italicized lower numerical values under the marker names indicate relative positions in this study and in USDA-MARC bovine genome map (www.animalgenome.org), respectively.

Table 2. Most likely position, likelihood-ratio test statistic values, and estimated effects of QTL for six growth and carcass quality traits in Hanwoo steers

Trait	cM[a]	LRT[b]	p-value[c]	h^{2d}	σ_Q^2/σ_p^{2e}	Marker close to QTL
Average daily gain	35	4.95	0.026	0.29	0.15	BMS518 (34.3 cM)
Final weight	63	4.44	0.035	0.22	0.11	BM4621 (62.5 cM)
Carcass weight	63	2.20	0.138	0.13	0.06	BM4621 (62.5 cM)
Backfat thickness	1	3.88	0.049	0.61	0.30	IL90 (0 cM)
Longissimus dorsi muscle area	67	2.94	0.086	0.24	0.12	BM4621 (62.5 cM)
Marbling score	140	0.94	0.332	0.63	0.05	BM8124 (117.4 cM)

[a] The position at which the likelihood-ratio test statistic was maximized.
[b] Likelihood-ratio test statistic, Ho: no QTL model ($\sigma_v^2 = 0$) vs. Ha: one QTL model ($\sigma_v^2 > 0$).
[c] p-values are chromosome-wise levels of linkage evidence.
[d] Narrow sense heritability. The proportion of phenotypic variance due to additive variance. (σ_u^2/σ_p^2).
[e] Proportion of phenotypic variance due to the QTL (v^2h^2).

phenotypic variance due to QTL allelic variance for the three QTL were 30%, 15%, and 11%, respectively. Because the average number of steers per sire or grandsire was small, sampling effects may have led to these effects being overestimated (Girgnola et al., 1996b). The QTL for LMA was detected with limited statistical support (p = 0.086 at chromosome-wise level), at 67 cM, close to BM4621, where the QTL for WT was detected. Also, the most likely position (63 cM) of the QTL for CWT was the same as the QTL for WT (Table 2 and Figure 1).

Several QTL studies have been conducted to find any associations with carcass quality traits in Hanwoo cattle (Kim et al., 2004; Cheong et al., 2008; Chung et al., 2008). However, most of the reports were based on candidate genes that are related to physiological functions with fat metabolism, and there was no report on candidate genes that were located on BTA6 (Cheong et al., 2007; Shin et al., 2007; Cho et al., 2008).

Previously, we reported a candidate gene study about a SNP, '12273_165' on BTA6 (Lee et al., 2008). The location of the SNP was near the marker ILSTS035, which was flanked by BM4621 and BMS2460 on BTA6 (www.animalgenome.org). We initially included the ILSTS035 maker (one of the twelve markers) to generate a BTA6 linkage map. However, there was no statistical significance of linkage for the marker, and the seven markers without ILSTS035 determined the BTA6 linkage map (results not shown). Lee et al. (2008) showed that the '12273_165' SNP was associated with WT, CWT, LMA, ADG at the 5% comparison-wise level, using the same population as this study. In similar or close to the SNP region, we also detected QTL for WT and ADG at the 5% chromosome-wise level, and LMA at the 10% chromosome-wise level (Table 2 and Figure 1). These results suggest that there is some evidence that alleles of causal genes responsible for growth and carcass traits are segregating in the Hanwoo population.

There are a few reports on the BTA6 region in which QTL were detected for the growth and carcass trait in this study (www.animalgenome.org). Kneeland et al. (2004) reported a QTL for average daily gain on feed in a composite line (Beefbooster M1). The QTL was detected in the region flanked by BMS382 and BMS1242, proximal to the ADG QTL in this study. Casas et al. (2000) reported a QTL for LMA and hot carcass weight in the region between BM3026 and BMS483, proximal to the QTL for LMA, WT and CWT in this study.

In this study, we first report QTL results from a chromosome-wise scan by applying a QTL interval mapping method in a population of Hanwoo cattle. However, to further refine the QTL position and to implement successive breeding schemes such as marker-assisted selection, a better structured pedigree is needed as well as high-marker density maps. Furthermore, the application of high throughput technologies such as bovine SNP chips to the detection of QTL by whole-genome wide association analysis would enable greater power to detect QTL and mapping precision (van Tassell et al., 2008).

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