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A Whole Genome Association Study to Detect Single Nucleotide Polymorphisms for Carcass Traits in Hanwoo Populations

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ABSTRACT : The purpose of this study was to detect significant SNPs for carcass quality traits using DNA chips of high SNP density in Hanwoo populations. Carcass data of two hundred and eighty nine steers sired by 30 Korean proven sires were collected from two regions, the Hanwoo Improvement Center of National Agricultural Cooperative Federation in Seosan, Chungnam province and the commercial farms in Gyeongbuk province. The steers in Seosan were born between spring and fall of 2006 and those in Gyeonbuk between falls of 2004 and 2005. The former steers were slaughtered at approximately 24 months, while the latter steers were fed six months longer before slaughter. Among the 55,074 SNPs in the Illumina bovine 50K chip, a total of 32,756 available SNPs were selected for whole genome association study. After adjusting for the effects of sire, region and slaughter age, phenotypes were regressed on each SNP using a simple linear regression model. For the significance threshold, 0.1% point-wise p value from *F* distribution was used for each SNP test. Among the significant SNPs for a trait, the best set of SNP markers were selected using a stepwise regression procedure, and inclusion and exclusion of each SNP out of the model was determined at the $p<0.001$ level. A total of 118 SNPs were detected; 15, 20, 22, 28, 20, and 13 SNPs for final weight before slaughter, carcass weight, backfat thickness, weight index, *longissimus dorsi* muscle area, and marbling score, respectively. Among the significant SNPs, the best set of 44 SNPs was determined by stepwise regression procedures with 7, 9, 6, 9, 7, and 6 SNPs for the respective traits. Each set of SNPs per trait explained 20-40% of phenotypic variance. The number of detected SNPs per trait was not great in whole genome association tests, suggesting additional phenotype and genotype data are required to get more power to detect the trait-related SNPs with high accuracy for estimation of the SNP effect. These SNP markers could be applied to commercial Hanwoo populations via marker-assisted selection to verify the SNP effects and to improve genetic potentials in successive generations of the Hanwoo populations. (**Key Words** : Single Nucleotide Polymorphism, Whole Genome Association, Carcass Traits, Hanwoo)

INTRODUCTION

Quantitative trait loci (QTL) mapping studies have been extensively performed and more than two thousand QTL for traits of economic importance have previously been reported in cattle (www.animalgenome.org). However, application of QTL to commercial populations via marker-assisted selection (MAS) has not been successful because the effects of the DNA markers that were detected and linked to causal mutations explained only a small portion of the total phenotypic variance for any trait (Sellner et al., 2007; van Eenennaam et al., 2007). Further, no appropriate or systematic approach was reported on how to efficiently integrate the DNA markers into the genetic evaluation systems in commercial populations (Druet et al., 2006).

Recently, rapid advancement of sequencing technology enabled whole genome sequencing of livestock species as well as human and experimental animals. The Bovine Genome Sequencing and Analysis Consortium et al. (2009) and Zmini et al. (2009) sequenced whole genomes, especially Hereford, and whole genome sequencing in other cattle breeds was also reported (Eck et al., 2009). From these studies, more than two million single nucleotide

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polymorphism (SNPs) have been found, which would provide huge number of DNA variation sources, expediting the identification of causal mutations for the genes underlying quantitative traits of interest in cattle (Sellner et al., 2007). In addition, high throughput technologies such as the development of bovine SNP chips enabled the genotyping of large amount of SNPs, e.g. the bovine 50k Illumina Infinium assay, which would enable genome-wide association (WGA) mapping to detect many QTL with high mapping resolution up to 1 or 2 Mb confidence region for a QTL position (Sellner et al., 2007; van Tassell et al., 2008; Sherman et al., 2010). This WGA method would take the place of the conventional QTL mapping approach based on linkage mapping using microsatellite markers, which is characterized as a mapping method with low mapping density and thus limited power for QTL detection (Sellner et al., 2007). For example, the number of reports and of the QTL for growth and carcass quality traits that have been detected in Hanwoo in the last two decades have been very limited, i.e. less than 20 QTL for a trait in the 20 publications (data not shown) because most of the QTL mapping approaches were based on candidate genes such that the categories of QTL to be detected were restricted within pre-chosen candidate genes with given physiological functions.

Here in, we first report WGA mapping results in Hanwoo cattle using high density SNP chips to find QTL for carcass quality traits.

MATERIALS AND METHODS

Animals and phenotypes

The Hanwoo data were collected from 289 steers in the two populations; i) steers (N = 186) of candidate bulls for progeny testing in the Hanwoo Improvement Center of National Agricultural Cooperative Federation in Seosan, Chungnam province and ii) the steers raised in commercial farms in Gyeonbuk province (N = 103). Both populations were composed of paternal halfsib pedigrees, the former from 20 Korean proven sires and the latter from 10 candidate bulls. The steers in Seosan were born between the spring and fall of 2006 and those in Gyeonbuk between fall 2004 and fall 2005. The steers were weaned at 5 or 6 months of age, and each group of 10 steers were raised in a pen. The feeding program was divided into early, middle, and late stage, at six month intervals. In the early and middle stages, the steers were fed with concentrates at 1.8% of body weight and *ad labium* in the late stage. The concentrates were composed of 15%, 13%, and 11% of crude protein, and 71%, 72%, and 73% totally digestible nutrients (TDN), in the respective feeding stages. Roughages with 4.5% crude protein and 37.5 TDN were offered *ad labium* with other additives such as vitamin and minerals. The steers in Gyeonbuk province were fed according to the feeding program of each farmer. Generally, they were weaned at 6 months of age, fed with the growth stage feed for 18 months, and given a high concentration diet in the last 6 months. Final weights (FWT) were measured before transport to the slaughter house. The average ages at slaughter were 723.7±20.6 d and 941.4±75.8 d for the steers in Seosan and Gyeongbuk, respectively. Twenty-four hours after slaughter, carcass weight (CWT) was measured and the 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. Carcass quality traits included in this study were backfat thickness (BFT), weight index (WtIndx), *longissimus dorsi* muscle area (LMA),

Table 1. Summary statistics for 289 observations on carcass quality traits in two Hanwoo populations

Trait	Region[a]	Average	Std Dev[b]	Minimum	Maximum	C.V.[c]
Final weight (kg)	Gyeongbuk	713.9	59.4	550	832	8.3
	Seosan	610.5	51.7	502	779	8.5
Carcass weight (kg)	Gyeongbuk	421.6	37.3	314	501	8.9
	Seosan	356.2	35.6	284	466	10.0
Backfat thickness (mm)	Gyeongbuk	13.4	5.2	3	30	39.1
	Seosan	10.0	4.1	1	21	40.9
Weight index	Gyeongbuk	64.4	3.7	53.0	71.1	5.8
	Seosan	66.8	3.0	58.6	73.6	4.5
Longissimus dorsi muscle area	Gyeongbuk	88.0	8.9	66	111	10.2
(cm^2)	Seosan	79.6	8.2	62	109	10.3
Marbling score (1-9)	Gyeongbuk	5.07	1.77	2	9	35.0
	Seosan	3.24	1.60	1	8	49.5

[a] Phenotypes were collected in two regions; the steers of the candidate bulls for progeny testing at the Hanwoo Improvement Center of the National Agricultural Cooperative Federation in Seosan, Chungnam province (N = 187) and the steers in commercial farms in Gyeongbuk province (N = 103). The average ages at slaughter were 723.7±20.6 d and 941.4±75.8 d for the former and latter populations, respectively.

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

marbling score (Marb). The Marb score was numbered as 1 thorough 9 according to the Korean Beef Marbling Standard (1 = trace, 9 = very abundant). Table 1 shows the summary statistics for the observed carcass quality traits from the steers of the two populations.

Molecular data

DNA samples from the 289 steers were prepared from blood according to standard protocols, and the DNA concentration was adjusted to 50 ng/μl. The Illumina bovine 50K SNP chips were used for WGA tests, in which 55,074 SNPs covering the bovine genome were included (Matukumalli et al., 2008; van Tassell et al., 2008). Approximately 200 ng of genomic DNA was used to genotype each sample on the Illumina Bovine SNP 50K Bead chip (Illumina, San Diego). Samples were processed according to the Illumina Infinium-II assay manual. Briefly, each sample was whole-genome amplified, fragmented, precipitated, and re-suspended in an appropriate hybridization buffer. Denatured samples were hybridized on the prepared bovine SNP 50 bead chip for a minimum of 16 h at 48°C. Following the hybridization, the bead chips were processed for the single-base extension reaction, stained, and imaged on an Illumina Bead Array Reader. Normalized bead intensity data for each sample were loaded into the Beadstudio 3.0 software (Illumina), which converted fluorescent intensities into SNP genotypes. SNP clusters for genotype calling were examined for all SNPs using Beadstudio 3.0 software.

Each of the 55,074 SNPs in the SNP chip was screened for the availability of GWA tests. First, SNPs in 29 autosomes were chosen, and any SNP was removed based on the following criteria: i) the number of genotype group is one or none (e.g. only AA genotypes and no AB or BB), ii) the minor allele frequency was smaller than 0.05, and iii) proportion of genotyped individuals was smaller than 90%.

Statistical analysis

Phenotypes were pre-adjusted using SAS GLM procedure of SAS v9.1 (SAS Inst., Inc., Cary, NC) before WGA tests. Two factors, i.e. region and sire, were fitted as a fixed and random effect, respectively, and a covariate for slaughter age was also fitted in the model. For BFT, WtIndx, LMA and Marb, the final weight was fitted as a covariate to detect SNPs that was associated with the post-slaughter measures, for which the effect was independent of body weight. The residuals were then regressed on each SNP using a simple linear regression model. The SNP genotype values were assigned as -1, 0, and 1 for *BB*, *BA* and *AA*, such that the allele substitution effect of replacing *B* with *A* allele was estimated. For significance threshold, 0.1% point-wise p value from *F* distribution was applied for each SNP test.

Due to the relationships of closely linked SNPs, which are called linkage disequilibrium (LD), i.e. non-random association between alleles of different SNPs, some of the significant SNPs would yield redundant information in implementing the MAS program. Thus, among the significant SNPs, the best set of SNP markers were selected for each trait using a stepwise regression procedure (Neter et al., 1990). Inclusion and exclusion of each SNP out of the model was determined at the $p<0.001$ level.

The variation explained by each SNP (S^2_{SNP}) was calculated as $\sum_{i}^{3}{}_{=1} \alpha_i^2 f_i - \mu^2$, where i indicates each genotype, α_i is allele substitution effect (= -â, 0, and +â for *BB*, *AB*, and *AA*, respectively, in which â is estimated from the simple regression analysis for the SNP), f_i is the frequency of i^{th} genotype, μ is the population mean that can be expressed as $(f_{AA}-f_{BB})$â (Falconer and Mackay, 1996). Proportion of phenotypic variance due to the SNP was then estimated as S^2_{SNP}/S^2_P, in which S^2_P was obtained from residual values of the trait after adjusting for fixed and random effects. Therefore, the estimate of the proportion of phenotype variance due to all of the significant SNPs was $\sum S^2_{SNPi}/S^2_P$. Another estimate was also used; $(RSS_{Ho}-RSS_{Ha})/RSS_{Ho}$, where the *RSS* is residual sum of squares, and *Ho* and *Ha* indicate the null and full model without and with fitting all significant SNPs, respectively (Neter et al., 1990).

RESULTS AND DISCUSSION

The steers from Gyeongbuk produced heavier, fatter, and more marbled carcasses with larger muscle area, because the steers were fed more than six months longer than those in Seosan. However, coefficients of variation were greater for all of the carcass traits in Seosan steers except WtIndx (Table 1). This indicates that more variability exits in carcass traits at approximately 24 months (the finishing stage of growth) than at 30 months of age (feeding with high concentration diets), suggesting that the variability at the former growth stage provide phenotype characteristics to be favorably exploited for QTL mapping studies.

Among the 55,074 SNPs in the Illumina bovine chips, a total of 32,756 SNPs (59.5%) were selected for WGA tests. The number of available SNPs and average interval sizes between flanking SNPs are shown in Table 2. More than one thousand SNPs were available in BTAs of 1 through 15, and BTA20. BTA1 contained the most SNPs (2,202) among the bovine chromosomes, which was proportionate to chromosomal length (Table 2). The physical maps using all of the available SNPs, spanned 2,543 Mbps, and the average distance between two adjacent SNPs was 77.6±75.0 kb. The intervals flanked by adjacent SNPs were generally

Table 2. The numbers of available SNPs in Hanwoo populations and average interval distance between flanking SNPs across all 29 autosomes (BTA)

BTA	Number of SNPs[a]	Average Interval size (kb)	Standard deviation (kb)	Total distance[b] (bp)
1	2,202	73.1	70.5	161,021,443
2	1,764	79.7	87.4	140,672,837
3	1,650	77.5	80.0	127,908,628
4	1,623	76.5	71.6	124,125,393
5	1,349	93.3	98.0	125,804,606
6	1,696	72.2	72.6	122,509,741
7	1,426	78.6	74.5	112,064,214
8	1,536	76.1	68.8	116,938,580
9	1,330	81.3	79.1	108,072,258
10	1,351	78.6	89.3	106,203,562
11	1,397	78.9	75.7	110,171,704
12	1,037	82.2	85.8	85,277,437
13	1,110	76.0	64.6	84,344,188
14	1,147	70.9	61.2	81,323,941
15	1,066	79.4	73.7	84,598,266
16	988	78.7	79.2	77,735,267
17	987	77.5	79.4	76,454,248
18	823	80.0	87.5	65,851,253
19	877	74.4	62.7	65,213,967
20	1,026	73.2	67.6	75,122,657
21	891	77.6	77.8	69,171,299
22	794	77.9	62.5	61,825,381
23	705	75.6	66.4	53,292,253
24	838	77.5	68.4	64,945,341
25	631	69.5	60.0	43,857,883
26	664	77.9	68.6	51,726,097
27	631	77.2	91.4	48,726,296
28	566	81.3	72.8	46,020,950
29	651	79.2	77.3	51,590,377
	Total: 32,756	Average: 77.6	Average: 75.0	Total: 2,542,570,067

[a] Among the 55,074 SNPs in the Illumina bovine 50K chip, SNPs for whole genome association (WGA) tests were selected if the number of genotype group is two or three, the minor allele frequency was greater than 0.05, and proportion of genotyped individuals was greater than 90%.

[b] The distances between the first and the last SNPs located on their respective chromosomes. The positions of the SNPs were based on the bovine reference genome (btau4.0).

similar across whole chromosomes ranging between 70 and 80 kb, except in BTA5 (93.3±98.0 kb). However, the number of available SNPs was quite small, i.e. less than 60% of the SNPs embedded in the Illumina SNP chip, and correspondingly, the average interval size of 77.6 kb was much larger than the expected value of approximately 60 kb. One of the main reasons may be ascertainment bias, i.e. the SNP discovery in building the Illumina chip was conducted using European breeds such as Hereford or Holstein (van Tassell et al., 2008), while Hanwoo breed have quite different genetic characteristics, because the breed has been domesticated and evolved on the Korean peninsula (Decker et al., 2009). Therefore, quite a few SNPs in the chip would have significantly different allele frequencies in Hanwoos compared to European breeds. Also, our unpublished results when using the same samples in Decker et al. (2009) indicated that the proportion of SNPs with minor allele frequency less than 5% was much greater in Hanwoos, e.g. 27.6%, compared to 22-23% in Angus, Hereford, and Holstein breeds.

A total of 118 SNPs were significantly associated with carcass traits at the $p<0.001$ level; 15, 20, 22, 28, 20, and 13 SNPs for FWT, CWT, BFT, WtIndx, LEA, and Marb, respectively (results not shown). Among the significant SNPs, the best set of 44 SNPs were determined after considering LDs between closely linked SNPs by the stepwise regression procedures; 7, 9, 6, 9, 7, and 6 SNPs for their respective traits (Table 3). Each set of SNPs for each trait explained 20-40% of phenotypic variance, with the

Table 3. Identities, positions, and effects of the SNPs associated with carcass traits with statistical significance at the point-wise 0.001 level in Hanwoo populations

Trait/SNP Marker[a]	SNP[b]	BTA	Kbp[c]	MAF[d]	-log$_{10}$P[e]	Estimate[f]	SE[g]	% σ^2_p [h]
Final weight (kg)								30.8 (28.9)
BTA-49198-no-rs	C/T	2	124,644	0.33	3.34	-14.37	3.55	4.5
BTB-00211171	A/G	4	114,679	0.24	4.12	17.14	4.27	4.6
BTA-62321-no-rs	C/T	10	32,294	0.43	3.20	12.11	3.56	3.3
BTB-02007301	C/T	11	12,225	0.47	3.41	-15.22	3.33	5.7
ARS-BFGL-NGS-8701	A/C	12	73,031	0.42	4.10	16.58	3.68	5.5
ARS-BFGL-NGS-11968	C/T	18	11,363	0.39	4.38	-15.61	3.56	5.1
Hapmap43308-BTA-61764	C/T	26	45,409	0.27	3.23	-11.50	4.12	2.2
Carcass weight (kg)								39.4 (36.5)
ARS-BFGL-NGS-28503	A/G	2	60,435	0.40	3.70	8.00	2.13	3.5
BTA-49198-no-rs	C/T	2	124,644	0.33	4.07	-9.24	2.19	4.5
BTB-00211171	A/G	4	114,679	0.24	3.94	12.12	2.61	5.5
BTB-01908971	G/T	8	53,370	0.22	3.69	-10.91	2.54	4.5
BTA-62321-no-rs	C/T	10	32,294	0.43	3.08	9.05	2.19	4.4
BTB-02007301	C/T	11	12,225	0.47	3.32	-9.32	2.05	5.2
ARS-BFGL-NGS-8701	A/C	12	73,031	0.28	4.19	10.54	2.28	4.6
ARS-BFGL-NGS-11968	C/T	18	11,363	0.39	3.94	-9.77	2.20	4.8
ARS-BFGL-NGS-15995	C/T	19	59,362	0.37	3.93	-7.18	2.28	2.5
Backfat thickness (mm)								23.1 (26.3)
BTB-00053769	A/G	1	122,462	0.29	3.53	1.08	0.30	3.5
ARS-BFGL-NGS-22578	A/G	5	1,879	0.48	3.16	0.99	0.29	3.2
ARS-BFGL-NGS-29931	A/G	5	39,554	0.17	3.55	-1.30	0.35	3.6
ARS-BFGL-NGS-100195	C/T	5	121,557	0.34	3.17	1.05	0.31	3.2
BTA-82147-no-rs	A/G	8	89,289	0.19	4.49	-1.48	0.35	4.7
Hapmap57754-ss46526149	A/G	26	51,726	0.48	4.70	-1.23	0.29	4.9
Weight index								38.8 (40.0)
BTB-00053769	A/G	1	122,462	0.29	4.76	-0.97	0.19	6.5
ARS-BFGL-NGS-102312	A/G	5	111,810	0.50	3.13	0.61	0.18	2.7
Hapmap59369-rs29018333	A/G	6	33,989	0.25	3.54	-0.88	0.20	4.8
ARS-BFGL-NGS-70430	A/C	8	32,563	0.49	4.26	0.79	0.17	5.0
BTA-82147-no-rs	A/G	8	89,289	0.19	3.91	0.72	0.22	2.6
ARS-BFGL-NGS-74013	C/T	12	31,009	0.37	4.08	0.75	0.19	4.0
ARS-BFGL-NGS-96997	C/T	19	55,248	0.33	5.00	-0.90	0.19	5.4
ARS-BFGL-NGS-91002	A/G	21	68,519	0.49	4.21	0.64	0.17	3.2
Hapmap57754-ss46526149	A/G	26	51,726	0.49	3.81	0.80	0.18	4.6
Longissimus dorsi muscle area (cm^2)								29.5 (29.4)
DPI-50	A/G	1	21,957	0.30	3.33	-1.79	0.49	3.7
BTA-114356-no-rs	G/T	4	11,166	0.29	3.50	2.10	0.51	4.8
Hapmap47240-BTA-30899	C/T	12	76,683	0.20	3.34	2.06	0.58	3.5
ARS-BFGL-BAC-18590	A/T	14	65,510	0.32	3.88	-2.03	0.48	4.9
ARS-BFGL-NGS-57555	C/T	14	74,617	0.47	3.45	-1.66	0.45	3.9
ARS-BFGL-NGS-52594	C/T	22	23,724	0.33	3.05	-1.70	0.48	3.5
ARS-BFGL-BAC-47334	A/G	24	21,639	0.26	4.31	-2.16	0.50	5.1
Marbling score (1-9)								21.9 (24.6)
Hapmap43396-BTA-89742	C/T	1	45,412	0.25	4.44	0.588	0.125	6.0
BTB-00069859	C/T	1	150,546	0.47	3.01	-0.315	0.105	2.5
BTB-01397485	C/T	11	34,733	0.50	4.27	0.454	0.111	4.6
Hapmap52669-rs29014752	A/C	15	20,459	0.36	4.05	0.452	0.114	4.5
ARS-BFGL-NGS-40665	A/G	17	11,187	0.24	3.32	-0.371	0.132	2.2
ARS-BFGL-NGS-10234	A/G	29	15,404	0.10	3.61	-0.513	0.113	2.1

[a,c] SNP marker annotations and their positions were based on the bovine reference genome (btau4.0).

[b] Nucleotides of substitution. [d] Minor allele frequency.

[e] Negative logarithm of the comparison-wise p-value of the test statistic against the null hypothesis of no SNP effect at the SNP position.

[f] Estimate is for the allele substitution effect of replacing the latter with the former allele (nucleotide) in the SNP column.

[g] Standard error.

[h] Proportion of phenotypic variance explained by the SNP. The values on the rows of trait are the sum of the % σ^2_p values of all SNPs, and the values in parenthesis are estimates using $(RSS_{Ho}-RSS_{Ha})/RSS_{Ho}$, where the *RSS* is residual sum of squares, and *Ho* and *Ha* indicate the null and full model without and with fitting all significant SNPs, respectively.

greatest value of 39.4% for CWT and the smallest value of 21.9% for Marb. All of the detected SNPs individually explained small portions of each trait's phenotypic variance ranging from 2.1% for the Marb SNP on BTA29 to 6.5% for WtIndx SNP on BTA1 (Table 3).

The SNPs detected in each trait were distributed in different chromosomes. When the SNPs were detected on the same chromosome, their locations were distant, e.g. at least 40 Mb, except the two LEA SNPs on BTA14 that were positioned at 66 and 75 Mbp (Table 3). These results were not surprising because the stepwise regression procedure applied excluded closely linked SNPs near the significant SNP. For example, two BFT SNPs that were located at 81.8 Mb and 89.3 Mb on BTA8 were detected ($p<0.001$). However, the two SNPs were closely linked to the BFT SNP, 'BTA-82147-no-rs', that was detected at 89.3 Mb with a greater statistical support than the two SNPs, so the two SNPs were removed in the model selection process (Table 3 and Figure 1).

The number of detected SNPs for each trait was not great compared with other WGA reports (Barendse et al., 2007; Sherman et al., 2010). This may be partly due to the genetic characteristics of the Hanwoo breed as well as the small sample size (N = 289). Unlike western European breeds such as Angus, Herford and Holstein, there has not been an intensive selection program implemented in the last half century in Hanwoo. Thus, LD between closely located markers would be much weaker in Hanwoo cattle, which would not allow for the detection of trait-related SNPs unless the SNPs are very closely located and tightly linked to causal mutation for the trait. We investigated how strong the LDs were between linked SNPs. To measure the magnitude of non-random association between SNPs, haplotypes were constructed for all steers using MERLIN program (1.1.2 version) (Abecasis et al., 2002), and then maternal chromosomes of the 289 steers were used to measure r^2, an LD statistic, between all pairs of syntenic SNPs in all autosomes (Devlin and Risch, 1995). Our results indicated that when two linked SNPs were located within 10 kb, the average r^2 value was 0.52. As the interval between two SNPs increased by 10 kb increments, the average r^2 values were 0.19 (20 kb), 0.24 (30 kb), 0.19 (40 kb), 0.16 (50 kb), 0.14 (60 kb), 0.12 (70 kb), 0.10 (80 kb, 90 kb), and less than 0.1 (>100 kb), respectively. These results showed that the LDs between linked SNPs were much weaker in Hanwoo compared to other western commercial breeds. For example, in Holstein cattle, the average r^2 value was 0.35 when two DNA markers were 50 kb apart, more than double the LD value in Hanwoo (Goddard et al., 2006). Also, the average distance between two flanking SNPs was 77.6 kb when using the available SNPs in the Illumina bovine chip in Hanwoo population (Table 2), with the average r^2 value less than 0.12. This result suggests that many QTL responsible for traits in this study would not be captured, unless the QTL are closely located to the SNPs on WGA tests. One solution would be to use chips containing much more SNPs than those in this study.

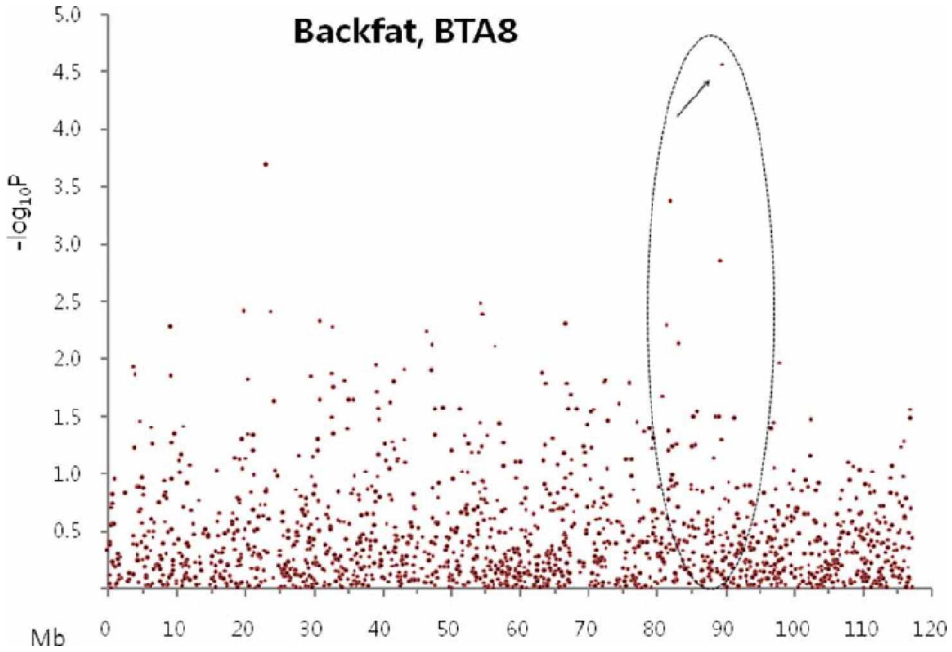

Figure 1. Test statistic (LRT) profiles for chromosome (BTA8) for backfat thickness. The arrow indicates the position (89,289k bp) of the SNP, 'BTA-82147-no-rs', that was associated with the trait with the most statistical significance in the chromosome. Two other SNPs affecting the trait with statistical significance ($p<0.001$ or $-\log_{10}P > 3.0$) are closely located to 'BTA-82147-no-rs' (inside the dot lined circle).

We did not apply chromosome- or genome-wise threshold values to take multiple tests into account to detect significant SNP for the carcass traits. Because there were more than 30,000 tests performed for each trait with a small sample size (N = 289), the threshold values that were obtained by permutation tests or false discovery rate methods (Churchill and Doerge, 1994; Benjamini and Hochberg, 1995), were so high that almost no SNP was concluded to be associated with the traits with statistical significance (results not shown).

There are some reports in which QTL were detected in the similar regions to where the trait-associated SNPs were detected in this study. Kim et al. (2003) detected two CWT QTL on BTA2 and BTA18 in a backcross F2 population from Angus and Brahman. The most likely positions (closely linked markers) were 19.7 cM (TGLA61) and 37.2 cM (BR4406), respectively. This study detected two CWT SNPs at 60 Mb and 11 Mb on their respective chromosomes (Table 3). In a region similar to where the CWT SNP was detected on BTA18 in Hanwoo, Casas et al. (2003) found a hot carcass weight QTL in a family crossed by a Brahman×Hereford sire and *Bos taurus* dams. Casas et al. (2003) also found one QTL for hot carcass weight on BTA10, and Takasuga et al. (2007) detected two QTL for live weight and three CWT QTL on BTA10 near a marker, DIK2014 (54.4 cM), in a purebred Japanese Black cattle population. Their QTL locations were close to the CWT SNP, 'BTA-62321-no-rs', in this study. Casas et al. (2000) and Li et al. (2004) reported a BFT QTL on BTA5 near the markers, BMS1248 (62 cM) and BMS490 (69 cM), in composite and *Bos taurus* populations, respectively. In the similar region, a BFT SNP, 'ARS-BFGL-NGS-29931', was detected at 39.5 Mb in this study. Casas et al. (2000) also reported a WtIndx QTL near a marker, BMS1248 (62 cM) on BTA5. We detected a WtIndx SNP at 111.8 Mb on the same chromosome. Casas et al. (2004) detected another WtIndx QTL on BTA26 near a marker, BLI040 in a Brahman x Angus sired population. In the similar region, a SNP, 'Hapmap57754-ss46526149' (51.7 Mb), was detected for the same trait in this study.

As SNP genotyping costs with advanced high-throughput technologies declined, the application of the SNP chip to commercial livestock populations became available (Hayes et al., 2009). If the SNPs in the chip covers a whole genome, and therefore have LD with all possible QTL for a trait, breeding values based on all of the available SNPs would be predicted and applied in genetic improvement programs. This new genetic evaluation system called genomic selection (GS), another form of marker-assisted selection, selects candidate bulls based on the genomic breeding values (GEBV) that are predicted from all of the SNP information, rather than based on pedigrees and phenotype information. The GS methodologies can be applied to our data in this study. However, the sample size of the data was too small to obtain GEBV with high accuracy (Hayes et al., 2009). Additional data of phenotypes and genotypes from SNP chips is being collected to detect more SNPs related to carcass traits, and to obtain estimates of the SNP effects with high accuracy.

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