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Analysis of Microsatellite Markers on Bovine Chromosomes 1 and 14 for Potential Allelic Association with Carcass Traits in Hanwoo (Korean Cattle)

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ABSTRACT : This study was conducted to investigate potential effects of previously identified QTL regions on carcass traits in Hanwoo. The data analyzed in this study was collected from 326 steers of 67 proven sire. Thirteen micorsatellite markers spanning QTL regions on bovine chromosomes 1 and 14 were genotyped in 326 steers. The following breeding values were analyzed for QTL effects. Cold carcass weight breeding value (CCWBV), longissimus muscle area breeding value (LMABV), marbling score breeding value (MSBV) and backfat thickness breeding value (BFTBV). Chi-square tests were performed to compare frequencies of individual allele between high and low breeding value groups. Significant differences of allele frequencies in BMS711, MCM130, BMS4049, and BMS2263 were found. And also, in RM180, BL1029, BM4305, and BMS2055 there were significant differencies of allele frequencies. These results showed a potential application for investigation of putative QTL locations. (**Key Words :** Microsatellite, Carcass Traits, Hanwoo)

INTRODUCTION

Association between molecular marker and phenotypic data is an important tool to expedite genetic improvement in agricultural species. (Xu et al., 2005) Genome researches in farm animals have discovered major genes and markers that can be utilized for marker assisted selection (MAS) in breeding programs (Dekkers, 2004). Several bovine chromosomal regions (or quantitative trait loci) have been successfully characterized for significant associations with economically important quantitative traits in some cattle crosses (Stone et al., 1999; Li et al., 2002; MacNeil and Grosz, 2002; Casas et al., 2003; Kim et al., 2003, Yeo et al., 2004).

Although some of the previously identified QTL effects might vary between cattle breeds, application of the QTL information for genetic improvement of Korean cattle (Hanwoo) can be a useful approach because mapping of Hanwoo QTL using current QTL methods requires well-defined mapping pedigrees, but development of such Hanwoo cattle pedigrees is extremely expensive and difficult under current commercial conditions in Korea (Kim et al., 2004)

We have previously studied microsatellite markers to use for individual identification and traceability of Hanwoo cattle (Yoon et al., 2005). In this study, we hypothesized that substantial difference in microsatellite allelic frequencies will exist between the low and high breeding value (BV) groups of Hanwoo cattle. Therefore, allelic association of microsatellite markers with important economic traits can be detected by comparing the allelic frequencies between the high and low BV groups of Hanwoo cattle. The most exciting advantage of this method is the possibility of detecting useful chromosomal regions responsible for heritable QTL without precise pedigree information. Several QTL studies have reported that microsatellite markers of bovine chromosomes 1 and 14 are associated with carcass traits (Kim et al., 2003; Kneeland et al., 2004).

In this study, the segregation of these associations in Hanwoo cattle was examined with 13 microsatellite markers on those two chromosomes and a large allelic frequency difference of 0.2 or greater, converting to phi coefficients of 0.22, in two independent Hanwoo sample

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Table 1. Summary statistics for phenotypic data

	Overall mean	Standard deviation	Median
CCW	308.45 kg	32.20 kg	307.50 kg
LMA	75.10 cm^2	8.08 cm	75.00 cm^2
MS	2.28	1.32	2.00
BFT	0.71 cm	0.29 cm	0.60 cm

CCW: cold carcass weight, LMA: longissimus muscle area.
MS: marbling score, BFT: backfat thickness.

Table 2. Summary statistics for breeding value of carcass traits

	Overall mean	Standard deviation	Median
CCWBV	0.61	10.14	0.00
LMABV	0.32	3.16	0.41
MSBV	0.02	0.56	-0.07
BFTBV	0.02	0.37	-0.02

sets would lessen the risk of false positive association results. The power of such allelic association is also largely depending on population-wide linkage disequilibrium of marker alleles with QTL, so most frequent microsatellite marker alleles across two independent Hanwoo populations were used to compare their frequencies between high and low BV groups.

MATERIAL AND METHODS

Animals and traits

Data was collected from progeny test at two stations (Korean Cattle Improvement Center as a branch of an Agricultural Co-operative Federation and the National Livestock Research Institute in Rural Development Administration) of Korea. A total of 326 blood samples of each steers from 67 paternal half-sib families were genotyped for 13 microsatellite loci on chromosome 1 and 14.

All carcass traits were evaluated following a 24-h chill postmortem by four carcass graders of APGS(Animal Products Grading Services), and factors used to determine APGS quality grades (marbling score) and yield grades (cold carcass weight, backfat thickness and eye muscle area) were recorded (Table 1).

The breeding values of the traits were predicted by multiple trait animal model using MTDFREML (Boldman et al., 1995). The analyzed model was;

$$y_{ijkl} = \mu + YS_i + L_j + bD_{ijkl} + a_{ijkl} + e_{ijkl}$$

where y_{ijkl} is the observation of the ith year season and jth location of birth for the lth trait; μ is the population mean; YS_i is the effect of year season; L_j is the effect of birth location; D_{ijkl} is the covariate of slaughter day; b is the coefficient of regression; a_{ijkl} is the random direct additive genetic effects; and, e_{ijkl} is the random residual error. The summary of the breeding values for the carcass traits were shown in Table 2.

PCR and genotyping

PCR was conducted with a final volume of 10 μl, including 1 μl of 10 X reaction buffer (10 mM Tirs, pH 8.3, 50 mM KCL, 0.1% Triton X-100, 1.5 mM $MgCL_2$), 0.7 μl dNTP Mix (2.5 mM), 10 pM of each primer, 20 ng of genomic DNA, and 0.5 U of Taq polymerase. Amplification of PCR products was carried out using a standard PCR program with 5-min denaturation at 94°C, 30 cycles for 30-sec at 94°C, 30-sec annealing at 55-65°C, 1-min extension at 72°C, and final extension for 10-min at 72°C. PCR product 1 μl were mixed with 0.5 μl GS - 400 TAMRA size standard (DNA fragments of known size labeled with ABI PRISM dye *N, N, N*[1]-tetra-methyl-6-carboxy-rhodamine (TAMRA)) (Perkim-Elmer, USA), 8 μl loading formamid solution. The samples were denaturized by heating at 90°C for 5-min followed by cooling on ice. Analyses of PCR products were performed by ABI 3100 Genetic Analyzer (Applied Biosystems, USA). Relative ratios of the detected virus sequences were determined by comparison of peak

Table 3. Allelic size distribution (bp) of the microsatellite markers used in this study

Chromosome	Marker	Allele										
		1	2	3	4	5	6	7	8	9	10	11
BTA1	BMS711	106	108	110	112 [1]	114 [2]	116	120	122			
	MCM130	105	113	115 [1]	117	119	121 [2]	123	127			
	BMS4032	92	94[1]	96	100	102	104	106 [2]	108	110		
	BMS4049	92	100[1]	102 [2]	104	106						
	BMS2263	152	154	156 [2]	158 [1]	160	162	166				
	URB014	115 [2]	117 [1]	121	123							
BTA14	BMS1747	85	89	91	93 [1]	95 [2]	97					
	RM180	119	123[2]	125	127	129 [1]	131	133				
	BL1009	155	161	165	167	169 [2]	171	173[1]	175	177	179	
	BL1029	144	148	150	152	154 [1]	160	162	164	166 [2]	168	170
	BMS4305	147	149	151	153	155	157	159	161	163 [1]	165 [2]	169
	BMS2055	149	153	157 [1]	159 [2]	161	163	165	167			
	BM6425	165	169	175	177	179 [1]	181	183	187	189	191 [2]	193

[1] Allele of the highest frequency. [2] Allele of the second highest frequency.

Table 4. Allele frequencies of BTA 1 and 14 microsatellite markers

Chromosome	Marker	Allele size	Frequency
BTA1	BMS711	112	0.4824
		114	0.3235
	MCM130	115	0.3866
		121	0.2268
	BMS4032	94	0.4236
		106	0.1700
	BMS4049	100	0.6623
		102	0.1722
	BMS2263	158	0.6051
		156	0.3462
	URB014	117	0.5965
		115	0.1634
BTA14	BMS1747	93	0.4571
		95	0.1540
	RM180	129	0.3431
		123	0.2606
	BL1009	173	0.2960
		169	0.2289
	BL1029	154	0.3584
		166	0.2035
	BMS4305	163	0.4094
		165	0.1287
	BMS2055	157	0.5482
		159	0.2500
	BM6425	179	0.2362
		191	0.2161

area values fork each of the detected fragments. Two alleles with highest allele frequencies for each marker were used for analysis and those two alleles were listed in Table 3 and their frequencies are in Table 4.

Statistical analysis

Allelic association analyses were conducted with common microsatellite alleles using SAS (SAS Inst. Inc., Cary, NC). For analysis of allelic association with BV, allelic frequencies were compared between high and low BV groups. Significant differences of allelic frequencies were determined from 2×2 contingency tables (high vs. low BV groups×with vs. without particular allele) in terms of chi-square. To maximize the sample size for allelic frequency comparison, medium breeding values were used to divide the high and low BV groups.

RESULTS AND DISCUSSION

A total of 13 polymorphic microsatellite markers on bovine chromosomes 1 and 14 were used for allelic association test with carcass characteristics in Hanwoo cattle. Table 3 presents allelic distribution of the 13 DNA markers in Hanwoo populations. The number of individual marker alleles was varied from 4 to 11. These common alleles were used to compare allelic distribution between high and low BV groups which incorporates BV of carcass traits. In addition, chi-square tests were performed to determine significant frequency differences between the two groups (Table 5).

For bovine chromosome 1, six significant allelic associations were found with carcass BV in the population. One significant allelic frequency differences with microsatellite marker BMS711 were found for marbling score BV in Hanwoo populations (Table 5). Two significant allelic frequency differences with microsatellite marker MCM130 and BMS 4049 were found for cold carcass BV and two significant allelic frequency differences with microsatellite marker BMS4049 and BMS2263 were found for longissimus muscle area BV. Only one allelic frequency difference in the marker BMS2263 was found for backfat thickness BV.

For bovine chromosome 14, five significant allelic associations were also found with carcass BV. Allele 1 of RM180 marker and allele 2 of BM4305 had significant association with longissimus muscle BV. Allele 1 of BL1029 and BMS2055 marker had significant association with marbling score BV. Allele 1 of BM4305 marker had significant association with backfat thickness BV.

In summary, our study attempted to identify QTL effects, which were previously detected, for their association with DNA markers. Microsatellite marker alleles within detected QTL regions were compared to find differences between

Table 5. Summary of Chi-square analyses of allelic frequency differences between high and low BV groups

Chromosome	Marker	Allele	Trait	Low	High	Chi-value
BTA1	BMS711	1	MSBV	0.55	0.45	4.4422*
	MCM130	1	CCWBV	0.4	0.6	4.5275*
	BMS4049	1	LMABV	0.56	0.44	5.7151*
		2	CCWBV	0.61	0.39	5.1562*
	BMS2263	2	LMABV	0.45	0.55	3.9515*
		2	BFTBV	0.58	0.42	6.7222**
BTA14	RM180	1	LMABV	0.57	0.43	5.5637*
	BL1029	1	MSBV	0.4	0.6	4.6488*
	BM4305	1	BFTBV	0.58	0.42	4.9760*
		2	LMABV	0.65	0.35	6.3431*
	BMS2055	1	MSBV	0.57	0.43	5.5754*

low and high breeding value groups for carcass traits. Significant allelic associations were found with carcass traits in our study, which indicates the potential QTL effects might be segregating in Hanwoo population.

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