Potential Allelic Association of Microsatellite Markers on Bovine Chromosome 5 with Carcass Traits in Hanwoo (Korean cattle)

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Received September 5, 2008 / Accepted September 23, 2008

A total of 10 polymorphic microsatellite markers on bovine chromosome 5 were used for allelic association tests with phenotypic characteristics in Hanwoo. The data analyzed in this study were collected from 326 steers. Chi-square tests were performed to compare the frequencies of individual alleles between the high and the low breeding value groups. The following breeding values were analyzed for QTL effects. The frequency of allele 239 of DIK2828 showed a significant difference between the high and the low breeding value groups in the breeding value of marbling score (MSBV). The allele 279 of BMC1009 was found to show significant differences in allelic distribution for the breeding value of cold carcass weight (CWBV) and the breeding value of backfat thickness (BFBV) and allele 285 showed significant differences in allelic distribution for CWBV, BFBV, and MSBV. The allele 200 of DIK4329 showed significant differences in allelic distributions for the breeding values of longissimus muscle area (LMABV) and BFBV. In this study, we identified the QTL for carcass traits at around 20 (DIK2828), 41 (BMC1009) and 95 (DIK4329) cM in chromosome 5. The results provided a useful reference for further positional candidate gene research and marker-assisted selection for fat metabolism and carcass traits.

Key words : QTL, microsatellite, allelic association, chromosome 5, carcass traits, Hanwoo

Introduction

Recent developments in molecular biology and in statistical methodologies for quantitative trait loci (QTL) mapping have made it possible to localize economically important QTL[1]. Identification and mapping of QTL for growth and carcass traits in beef cattle have recently been reported in a number of studies [3,5,9,14]. Several bovine chromosomal regions have been successfully characterized for significant association with economically important quantitative traits in some cattle crosses [4,7-9,12,14,17]. [3] reported that QTL were segregating in the regions of BTA [5-7,13,14,17,19,22,27,29]. for carcass composition and growth in a Piedmontese paternal half-sib family and in a Belgian Blue family.

In this study, we hypothesized that substantial differences in microsatellite allelic frequencies will exist between the low and high BV groups of Hanwoo. Therefore, allelic associations of microsatellite markers with important economic traits could be detected by comparing the allelic fre-

quencies between the high and low BV groups of Hanwoo. The most exciting advantage of this method is the possibility of detecting useful chromosomal regions responsible for heritable QTL without precise pedigree information. The segregation of these associations in Hanwoo was examined with 10 microsatellite markers on chromosome 5. The power of such allelic association is also largely dependent on population-wide linkage disequilibrium of marker alleles with QTL, so the most frequent microsatellite marker alleles across two independent Hanwoo populations were used in the comparison of their frequencies between the high and the low BV groups.

Materials and Methods

Animals and genetic evaluation of traits

Data were collected from two progeny testing stations (Korean Cattle Improvement Center, a branch of the Agricultural Co-operative Federation and the National Livestock Research Institute in Rural Development Administration) in Korea. A total of 326 blood samples of steers were genotyped for 10 microsatellite loci on chromosome 5 (Table 1).

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Table 1. The primer sequences of the microsatellite loci on chromosome 5 typed for in this study

Locus	Annealing Temp.	Primer sequence	Dye color
CA084	62°C	GGGAGGGGAAATTCTTTGCATTC GTGACTGGAGGTCTCAGCCT	NED
DIK4782	62°C	GCATCTGAGAGCCTCTTTGG TGCTTTTGCTTTTCATTCCTG	FAM
BMC1009	62°C	GCACCAGCAGAGAGGACATT ACCGGCTATTGTCCATCTTG	FAM
DIK4329	62°C	CCGGAGTGAGTGACCATTCT TGCTCCCTCTCTGGAAATA	JOE
DIK1048	63°C	CATGAGGTTGCAAAGACTGAG TGAGGAACCTTCACTTTCACTT	FAM
DIK5248	63°C	TGGATAGAGCCTTGAATGG TTCCAATGATGCAGGTCAA	JOE
DIK2400	63°C	GCTGGATGAAAATGAAAACAAG GGTGCAGGTAAGGAGAGGTG	NED
DIK2718	63°C	AGGAAGGACAAGGACATTGC AGAGGCTCAAAGGCTTAATGG	FAM
MNB-71	63°C	GTACATCTAAGGCAGAGCCAAC ACTCTCTCAGCTACAGCCCTTC	NED
DIK2828	63°C	TGGTGTCTGCCAAAGCAGTA CGGGAATTTTCAGAGGGAAT	FAM

All carcasses were evaluated following a 24-h chill post-mortem by four carcass graders of the APGS(Animal Products Grading Services), and the factors used to determine the APGS quality grades (marbling score) and yield grades (cold carcass weight, backfat thickness and longissimus muscle area) were recorded (Table 2).

The breeding values of the traits except dressing percentage were predicted by the multiple trait animal model using MTDFREML [2]. The analyzed model was as follows: $y_{ijkl} = \mu + YS_i + L_j + bD_{ijkl} + a_{ijkl} + e_{ijkl}$, where y_{ijkl} = the observation of the j th year season, j th location of birth; μ is the population mean; YS_i is the fixed effect of year season; L_j is the fixed effect of birth location; D_{ijkl} is the covariate of slaughter day; b is the coefficient of regression; a_{ijkl} is

Table 2. Summary statistics for the phenotypic data

Traits	Mean	Std.	Median
CW	308.45 kg	32.20	307.50
LMA	75.10 cm ²	8.08	75.00
MS	2.28 point	1.32	2.00
BF	0.71 mm	0.29	0.60

CW: cold carcass weight, LMA: longissimus muscle area, MS: marbling score, BF: backfat thickness.

the random direct additive genetic effect; and e_{ijkl} is the residual error. (Table 3)

PCR and genotyping

PCR was conducted with a final volume of 10 μ l, including 1 μ l of 10 $\times$ reaction buffer (10 mM Tris, pH 8.3, 50mM KCL, 0.1% Triton X-100, 1.5 mM MgCl₂), 0.7 μ l dNTP Mix (2.5mM), 10 pM of each primer, 20 ng of genomic DNA, and 0.5U 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 products of 1 μ l were mixed with 0.5 μ l GS - 400 TAMRA size standard (DNA fragments of known size labeled with ABI PRISM dye N, N, N'-tetra-methyl-6-carboxy-rhodamine (TAMRA) (Perkin-Elmer, USA) and, 8 μ l loading formamid solution. The samples were denatured by heating at 90°C for 5-min followed by cooling on ice. Analyses of PCR products were performed by using the ABI 3100 Genetic Analyzer (Applied Biosystems, USA). The relative ratios of the detected virus sequences were determined by comparisons of peak area values for each of the detected fragments.

Statistical analysis

Evaluations of the QTL regions on bovine chromosome 5 in young bull populations were performed using several statistical methods. Allelic association analyses were conducted with common microsatellite alleles using SAS (SAS Inst. Inc., Cary, NC). For the initial analysis of allelic association with BV, allelic frequencies were compared between the high and the low BV groups. Significant differences of allelic frequencies were determined from 2 $\times$ 2 contingency tables (high vs. low BV groups $\times$ with vs. without particular allele) by chi-square test. Other tests of 2 $\times$ 2 contingency tables such

Table 3. Summary statistics for the breeding value of the carcass traits

Traits	Mean	Std.	Median
CWBV	0.61	10.14	0.00
LMABV	0.32	3.16	0.41
MSBV	0.02	0.56	-0.07
BFBV	0.02	0.37	-0.02

CWBV: breeding value of cold carcass weight, LMABV: breeding value of longissimus muscle area, MSBV: breeding value of marbling score, BFBV: breeding value of backfat thickness.

as continuity-adjusted chi-square and Fisher's exact test were also performed. 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 10 polymorphic microsatellite markers on bovine chromosome 5 were used for allelic association tests with phenotypic characteristics in Hanwoo. The number of alleles, size range of alleles, polymorphism information content (PIC) and observed heterozygosity (H_o) and expected allelic diversity (Div) are given in Table 4. A total of 169 alleles were observed at the 10 loci in 326 individuals in Hanwoo. All the loci were polymorphic and the number of alleles ranged from 9 (CA084) to 28 (DIK2400) with an average value of 16.9. The highest polymorphic information content (PIC) value was DIK2400 (0.908) and the lowest was DIK2718 (0.603) with an average value of 0.699. The highest observed heterozygosity (H_o) value was 0.655 (DIK1048) and the lowest was 0.906 (DIK2400), with an average value of 0.772. The expected allelic diversity (Div) ranged from 0.653 (DIK2718) to 0.914 (DIK2400) and (the average value of Div was 0.797).

These common alleles were used to compare the allelic distributions between the high and the low BV groups by incorporating the BVs of carcass traits. In addition, chi-square tests were performed to determine significant frequency differences between the two groups. We analyzed the allelic distributions by using MS markers on chromosome 5 (Table 5) and found significant associations

Table 4. Characterization of microsatellites analyzed on chromosome 5 in Korean cattle (Hanwoo)

Loci	No of allele	PIC	H_o	Div
DIK2828	16	0.753	0.814	0.782
DIK2718	10	0.603	0.665	0.653
BMC1009	27	0.770	0.696	0.786
DIK4782	16	0.881	0.826	0.891
CA084	9	0.681	0.706	0.725
DIK5284	14	0.798	0.838	0.821
DIK1048	14	0.666	0.655	0.711
DIK4329	19	0.825	0.827	0.839
DIK2400	28	0.908	0.906	0.914
MNB-71	16	0.832	0.795	0.848

PIC: Polymorphic Information content, H_o : observed heterozygosities, Div: expected allelic diversity.

Table 5. Allele frequencies of microsatellite markers on bovine chromosome 5

Loci	Position (cM)	Allele	Freq.
DIK2828	20	239	0.362
		235	0.171
DIK2718	30	193	0.502
		195	0.285
BMC1009	41	279	0.413
		285	0.184
DIK4782	50	166	0.251
		172	0.168
CA084	56	130	0.375
		132	0.296
DIK5248	74	206	0.271
		198	0.211
DIK1048	87	116	0.421
		124	0.294
DIK4329	95	200	0.322
		222	0.136
DIK2400	108	246	0.148
		250	0.121
MNB-71	133	212	0.247
		208	0.229

with differences among four allelic distributions for three MS markers with BVs of carcass traits (Table 6).

The frequency of allele 239 of DIK2828 showed a significant difference between the high and the low breeding value groups in the BVs of marbling score ($p < 0.01$). Two alleles, 279 and 285 of BMC1009 showed significant associations with the BVs of carcass traits. Allele 279 was found to show significant differences in allelic distributions for CWBV and BFBV and allele 285 showed significant differences in allelic distributions for CWBV, BFBV, and MSBV. The allele 200 showed significant differences in allelic distribution for LMABV and BFBV.

In an earlier QTL scan involving a *Bos taurus* × *Bos indicus*

Table 6. Major allele frequencies in relation to economic traits on bovine chromosome 5

Loci	cM	Allele	Traits	Freq.	Freq. of pop.		χ^2
					Low	High	
DIK2828	20	239	MSBV	0.362	59	41	6.10**
BMC1009	41	279	CWBV	0.413	38	62	14.43**
			BFBV		37	63	15.87**
		285	CWBV	0.184	35	65	8.02**
			BFBV		40	60	3.41*
DIK4329	95	200	MSBV		42	58	3.82*
			LMABV	0.322	40	60	7.53**
			BFBV		27	73	13.47**

*: $p < 0.05$, **: $p < 0.01$

sire, the most significant statistical support for QTL on chromosome 5 were for the effects on percentage of bone in the wholesale rib and dressing percentage [14]. Also, [9] found associations between birth weight and haplotype segments on chromosome 5 in a commercial population of *Bos taurus* breed. [7] found birth weight QTL on chromosome 6 and 5 under the RA and LC models with significant and highly suggestive evidence for linkage, respectively. In this way, it was reported through much research that QTL for growth traits exist on chromosome 5. Much research about carcass trait and fat metabolism had also been done [4,7,10,15]. [15] reported that the major haplotype of the phosphodiesterase 1B (PDE1B) gene had significant association with less fat thickness. The PDE1B gene is located around 32 cM on chromosome 5. It was also reported that the major haplotype of the nucleolar antigen 1 (NOL1) gene had significant association with daily gain. The NOL1 gene is located around 108 cM. In this study, we identified significant differences in allelic distributions for carcass traits at around 20 (DIK2828), 41 (BMC1009) and 95 (DIK4329) cM on chromosome 5. Loci DIK2828 and BMC1009 were assumed to be associated with fat metabolism because there were significant differences in allelic distributions for MSBV and BFBV. This result was similar to the finding of [15]. The amount and distribution of fat had an important impact on carcass and meat quality in beef cattle [13]. Beef carcasses can be stratified by using subcutaneous fat thickness alone or in combination with marbling [16]. Breeding for optimal fat is therefore one of the major goals toward better profitability in the beef industry. The results provide a useful reference for further positional candidate gene research and marker-assisted selection for fat metabolism and carcass traits.

Acknowledgements

This study was supported by Technology Development Program for Agriculture and Forestry, Ministry for Agriculture, Forestry and Fisheries, Republic of Korea.

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초록 : Microsatellite 의 대립유전자 빈도를 이용한 한우의 경제형질과의 연관성 규명

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본 연구는 한우의 5번 염색체 내에 존재하고 있는 10개의 microsatellite marker의 대립유전자 빈도를 이용하여 한우의 경제형질과의 연관성을 지닌 좌위를 탐색하기 위하여 실시하였다. 한우 326두를 대상으로 10개의 유전좌위를 분석한 결과 총 169개의 대립유전자가 검출되었다. 모든 유전좌위에서 다형성이 검출되었으며 각 유전좌위별 대립유전자의 수는 9에서 28개로 나타났으며 평균 16.9로 나타났다. 가장 높은 PIC 값을 가진 유전좌위는 DIK2400(0.908)이었으며 가장 낮은 유전좌위는 DIK2718 (0.603)으로 검출되었다. 관측된 이형접합도에서는 DIK1048 (0.655)가 가장 높게 나타났으며, DIK2400 (0.906)은 가장 낮은 것으로 검출되었다. 분석된 MS marker들의 대립유전자의 빈도를 이용하여 경제형질과의 연관성을 탐색하기 위하여 각 경제형질별 육종가를 대상으로 상위그룹과 하위그룹으로 나누어 Chi-square검정을 실시하였다. 분석결과 DIK2828의 239 대립유전자는 근내지방도에서 상위그룹과 하위그룹의 빈도차가 유의적인 차이를 보이는 것으로 나타났다. BMC1009의 279 대립유전자는 도체중과 등지방두께에서 유의적인 차이를 확인하였으며 285대립유전자는 도체중, 등지방두께 그리고 근내지방도에서 유의적인 차이가 확인되었다. DIK4329의 200대립유전자는 등심단면적과 등지방두께에서 유의적인 차이가 확인되었다. 본 연구에서 유의적인 차이가 확인된 유전자좌위는 5번 염색체 내 20 (DIK2828), 41 (BMC1009) 그리고 95 (DIK4329) cM로 나타났다.