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Association of Polymorphisms in the Bovine Leptin Gene with Ultrasound Measurements for Improving in Korean Cattle

H. S. Kong[1, 2], J. D. Oh[2], S. G. Lee[2], Y. S. Hong[2], W. I. Song[2], S. J. Lee[2], H. C. Kim[3]
B. H. Yoo[4], H. K. Lee[2] and G. J. Jeon[2, *]
[1] Animal Products Grading Service, Korea

ABSTRACT : The identification method that inflects real time ultrasound (RUT) and the potential application of marker assisted selection (MAS) for improvement of a cow population of Hanwoo (Korean Native cattle) was studied. The averages of RUT longissimus muscle area, RUT fat thickness, and RUT marbling score scanned at the 13th rib were 55.78 cm^2, 3.70 mm and 3.83 scores, respectively. We investigated the effects of the two SNPs (*Kpn2 I* and *Msp I*) in the leptin gene on carcass traits for Hanwoo cows by using ultrasound measurements. Genotype CC of the *Kpn2 I* had a significantly higher effect on back fat thickness (4.23 mm) and longissimus muscle area (57.57 cm^2) than genotype TT (3.14 mm, 53.93 cm^2, respectively, $p<0.05$). Genotype AA of the *Msp I* had a significantly higher effect only on marbling score (5.37) than genotype AB (3.57, $p<0.05$) and BB (3.37, $p<0.05$). Significant effects of SNPs in the leptin gene were found for the ultrasound measures of body composition in live cattle. (**Key Words** : Korean Native Cattle, Leptin Gene, Improvement of Cow, Real Time Ultrasound (RTU))

INTRODUCTION

Genetic improvement of the domestic animals based on phenotypic information needs quite time demanding and is slower than expected. However, it has still been used in domestic animal breeding industry. Real time ultrasound (RTU) has been used to assess meat quality of live animals in beef cattle. The potential of this technology has been well reported by many authors (Herring et al., 1998; Moser et al., 1998; Wilson et al., 1999; Reverter et al., 2000; Devitt and Wilton, 2001). The RTU technology has contributed to shorten generation intervals for improvement of the domestic animals.

Leptin is a hormone secreted predominantly from white adipose tissue and performs important roles in the control of body weight, feed intake, immune function, and reproduction (Santos-Alvarez et al., 1999; Kadokawa et al., 2000; Block et al., 2001). Plasma leptin levels in cattle and sheep increase linearly with increased body fat mass and with increased energy balance (Blache et al., 2000; Delavaud et al., 2000; Ehrhardt et al., 2000; Baik and Kim, 2004). Systemic or central administration of leptin reduces feed intake in rodents, chickens, pigs, and sheep (Halaas et al., 1995; Barb et al., 1998; Raver et al., 1998; Henry et al., 1999) and while data from livestock species remains sparse, leptin appears to be an important component of a feedback loop involving key metabolic regulators including insulin, glucocorticoids and the sympathetic nervous system (Houseknecht et al., 1998). Polymorphisms in the human leptin gene were associated with low circulating leptin levels (Hager et al., 1998), birthweight (Orbak et al., 2001), and obesity (Ohshiro et al., 2000). Since the bovine leptin gene has been identified on chromosome 4, several SNPs have been previously identified in introns and exons of leptin among different breeds of cattle. Polymorphisms in the bovine leptin gene have been described (Pomp et al., 1997; Fitzsimmons et al., 1998; Haegeman et al., 2000; Yoon et. al., 2005) and an association with fat deposition in beef cattle was reported (Fitzsimmons et al., 1998). These genetic informations for fatty-phenotypic traits are valuable in breeding for high quality meat through marker assisted selection (MAS).

The objective of the present study was to determine genetic and phenotypic correlations of polymorphism in leptin gene with ultrasound measures of body composition in live cattle and also to identify the method that inflects

* Corresponding Author: G. J. Jeon. Tel: +82-31-670-5330, Fax: +82-31-675-5331, E-mail: gj_jh@hotmail.com
[2] Genomic Informatics Center, Hankyong National University, Korea.
[3] National Livestock Research Institute, RDA, Korea.
[4] Namyangju-city Agricultual Information System, Korea.
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Table 1. General performance on meat quality traits measured by ultrasound in Hanwoo cows

	No. of obs.	Mean±SD[1]	Min[2]	Max[3]
No. of cow	275			
Age (month)	275	40.61±15.49	18	88
Traits				
BF(mm)[4]	275	3.70±2.39	1.0	11.2
LMA(cm^2)[5]	275	55.78±9.97	31.2	95.6
MS(score)[6]	275	3.83±3.67	1.0	22.0

[1] SD = Standard deviation. [2] Min = Minimum value. [3] Max = Maximum value.
[4] BF= Back fat thickness. [5] LMA = Longissimus muscle area. [6] MS = Marbling score.

RTU and MAS for improvement of a caw population (Hanwoo).

MATERIALS AND METHODS

Animal

Animals used in this study were 275 Hanwoo (Bos taurus) cows from a farm in Namyangju city, Gyeonggi-Do, Korea.

Real-time ultrasound measure

Real-time ultrasonic scans were assessed from 275 cows born in a farm in Namyangju city. Real time Ultrasound were scanned for 275 cows before slaughter by skilled technicians. Ultrasound measurements were obtained from the SV-900 Scanner unit, which used the 15 cm, 3.5 MHz linear array transducer. This transducer allowed the entire longissimus muscle area (LMA) to be scanned in a single image. Single image by this equipment on 13th rib rump scanned were interpreted and assessed by an experienced technician. Data on individual longissimus muscle area and backfat thickness (BF) were obtained by assessment by way of imaging software program. Otherwise, data of marbling scores (MS) were obtained by comparing to standard references, which described the score according to degree of marbling, by an expert technician.

Genomic DNA extraction and PCR amplification

DNA samples were extracted from blood by some modification of the method used by Miller et al. (1988). The *Kpn2 I* (exon2) region of genomic DNA was amplified using PCR with the following primers: Forward, 5'-atgcgctgtggacccctgtatc-3' (48-69 nucleotides in the sequences: GenBank Accession. No. BT020625), Reverse, 5'-tggtgtcatcctggaccttcc-3' (121-141 nucleotides). The *Msp I* (exon3) region was amplified using PCR with the following primers: Forward, 5'-agcagtccgtctcctccaaacagag-3' (190-214 nucleotides in the sequences on: GenBank Accession. No. BT020625), Reverse, 5'-ggactttgggaagagaggccttca-3' (557-580 nucleotides). The Polymerase Chain Reaction was conducted in 10 μl volumes, each containing 100 ng of genomic DNA, 10× PCR buffer (100 mM Tris pH 8.9, 50 mM KCl, 15 mM MgCl, 0.01% gelatin, 0.1% Triton X-100, 10 mg/ml BSA), 10 pmole of each primer, 40 uM of dNTPs and 0.5 unit TaqDNA polymerase (Promega, USA). The condition of PCR was a first denaturation step of 5 min at 94°C followed by 30 cycles, each consisting of 40 sec at 94°C, 30 sec at 62°C (*Kpn2 I*) or 55°C (*Msp I*), 1 min at 72°C and then, a final step of 10 min at 72°C using PTC 200 peltier thermal cycler (MJ Research, USA).

Genotyping

PCR products of the K120A region were digested with *Kpn2 I* restriction enzyme(T^CCGGA) and separated on a 4% nuesive (TAKARA, Japen) gel. The uncut fragment represents the arginine variant, whereas the *Kpn2 I* RFLP fragments of 73 and 22 bp represent the cysteine variant. And the other PCR products (C542T) were digested with *Msp I* restriction enzyme(C^CG_G) and separated on a 4% nuesive gel. The uncut fragment represents the proline variant, whereas the *Msp I* RFLP fragments of 170 bp, 132 bp, 50 bp, 39 bp represent the proline variant.

Statistical analysis

The following linear covariant models were used for the analysis using the SAS.

$$Y_{ijk} = u+b_1Age_i+b_2Age_i^2+G1_j+e_{ijk}$$

$$Y_{ijk} = u+b_1Age_i+b_2Age_i^2+G2_j+e_{ijk}$$

where Y_{ijk} = a phenotypic record; u = overall mean; b_1, b_2 = regression coefficients; Age_i = fixed effect of age of cow considered as a covariate; Age_i^2 = fixed quadratic effect of age of cow considered as a covariate; $G1_j$, $G2_j$ = effect of RFLP genotypes; and, e_{ijk} = random residual.

RESULT AND DISCUSSION

Table 1 shows the mean, SD, minimum and maximum for each trait and age at slaughter. The average age of cows was 40.6 months and all animals tested in present study were scanned within 2 weeks. The average of RTU longissimus muscle area, RTU fat thickness, and RTU

Table 2. Genotype and allele frequency of the *Kpn2 I* and *Msp I* polymorphism on leptin gene in Hanwoo

Loc	Genotype	Frequency	Allele	Frequency
Kpn2 I	CC	0.262(72)	C	0.500
	CT	0.476(131)	T	0.500
	TT	0.262(72)		
Msp I	AA	0.294(81)	A	0.585
	AB	0.582(160)	B	0.415
	BB	0.124(34)		

Table 3. Effect of the polymorphism in leptin gene on economic traits in Hanwoo

Loc	Genotype	BF	LMA	MS
Kpn2 I	CC	4.23±0.32[a]	57.57±1.34[a]	4.02±0.49
	CT	3.60±0.60[ab]	55.23±1.14[ab]	3.40±0.41
	TT	3.14±0.35[b]	53.93±1.46[b]	3.86±0.53
Msp I	AA	3.83±0.54	57.29±2.21	5.37±0.70[a]
	AB	3.58±0.23	54.61±0.94	3.57±0.34[b]
	BB	3.84±0.34	56.68±1.49	3.37±0.50[b]

BF = Back fat thickness, LMA = Longissimus muscle area.
MS = Marbling score.
[a, b] Different superscripts within columns are significantly different ($p<0.05$).

marbling score scanned at 13th rib were 55.78 cm^2, 3.70 mm and 3.83 scores, respectively. In general for Korean bulls and steers, RTU measured carcass traits for over 20 month of age from Hanwoo steer would highly correlated (over 0.90) to the direct measured carcass traits (Kim et al., 2003).

Most studies on ultrasound measurements and their accuracies have been studied for bulls and steers but not much has been done for cows.

Leptin is a 16-kDa protein that is synthesized by adipose tissue and is involved in regulation of feed intake, energy balance, fertility, and immune functions (Fruhbeck et al., 1998; Houseknecht et al., 1998). Adipose tissue mass is influenced by volume and number of adipocytes (Prins and O'Rahilly, 1997). Adipocytes are also the principal site of leptin production in mice (Zhang et al., 1994), pigs (Mendiola et al., 1997), sheep (Dyer et al., 1997), cattle (Ji et al., 1998), and humans (Considine, 1997). Several polymorphisms within the leptin gene in cattle reported. A microsatellite has been located in the 5'UTR of the leptin gene (Wilkins and Davey, 1997) and nine SNPs have been discovered in intron 2 of the bovine leptin gene (Lein et al., 1997; Pomp et al., 1997). The polymorphisms in this study were *Kpn2 I* and *Msp I*. The *Kpn2 I* site located in the exon 2 of the leptin gene. And the *Msp I* site located in the exon 3. The SNP in exon 2 could be distinguished by digestion with the restriction enzyme *Kpn2 I* following amplification with a purposeful mismatch primer. Through electrophoretic analysis, the C allele was cleaved into two fragments of 75 and 19 bp, while the T allele was remained uncut at 94 bp (Figure 1). The SNP in exon 3 could be distinguished by digestion with the restriction enzyme *Msp I* following

Figure 1. A 5% polyacrylamide gel displaying a *Kpn2 I* restriction digest on an amplified portion of Hanwoo leptin exon 2. Lane 1, 100 bp ladder, lanes 2, 8 are CC, lanes 3, 4, 5, 10, 11 are CT, lanes 6, 7, 9 are TT.

Figure 2. A 3% agrose gel displaying a *Msp I* restriction digest on an amplified portion of Hanwoo leptin exon 3. Lane 1, 100 bp ladder, lanes 15, 16 are AA, lanes 2, 3, 4, 5, 9, 10, 11, 13, 14 are AB, lanes 5, 6, 7, 17 are BB.

amplification with a purposeful mismatch primer. The A allele was cleaved into three fragments of 170, 130 and 90 bp, while the T allele was of 170, 130, 50 and 40 bp (Figure 2). In our study, the PCR-RFLP analysis for *Kpn2 I* showed that the frequency of allele C and T were 0.5 and 0.5, respectively, and the frequency of genotype CC, CT and TT were 0.262, 0.476 and 0.262, respectively. Similarly in PCR-RFLP analysis for *Msp I*, we had 0.585 and 0.415 for A and B allele frequency, respectively, and 0.294, 0.582 and 0.124 for the AA, AB and BB genotype frequency, respectively.

We next investigated the effect of the two SNPs (*Kpn2 I* and *Msp I*) in the leptin gene on ultrasound measurements in Hanwoo cows. As shown in Table 3, *Kpn2 I* showed significant effects on back fat and longissimus muscle area. And *Msp I* showed a significant effect only on marbling score. The polymorphisms reported that *Kpn2 I* changed the amino acid in exon1 2 (Arg/Cys), which has been confirmed by Konfortov et al. (1999) and they also reported several other intronic polymorphisms in a diverse panel of cattle breeds. Buchanan et al. (2002) reported a significant

effects of polymorphism in exon 2 of leptin gene on grade fat and average fat (mean value of three measures of backfat thickness along the 12th rib), with the T allele associated with higher fat, but with no significant association with carcass marbling score, which is an antagonistic result compared with other studies (Buchanan et al., 2002). Schenkel et al. (2005) also reported that the two SNPs in the exon 2 of the leptin gene were associated with fat and lean yield and grade fat. In this study, as shown in Table 3, genotype CC of the *Kpn2 I* had a significantly higher back fat thickness (4.23 mm) and longissimus muscle area (57.57 cm^2) than genotype TT (3.14 mm, 53.93 cm^2, respectively, $p<0.05$), but with no significant association with marbling score. The *Msp I* located in exon 3 of leptin gene. This is a silent substitution on the change of amino acid. But the effect of the AA genotype on marbling score was significant ($p<0.05$)(Kim, 1997). In this study, genotype AA of the *Msp I* had a significantly higher marbling score (5.37) than genotype AB (3.57, $p<0.05$) and BB (3.37, $p<0.05$). Although a T allele of the *Kpn2 I* had not a significant effect on marbling score. It had a thinner effect in back fat thickness. An A allele of the *Msp I* was found a significant effect on high marbling score.

Bulls and steers only can yield carcass results of individual by slaughtering. However, cows can not easily obtain information of carcass traits due to continuous reproduction. Hence, if ultrasound measurements are applicable with considerable accuracies and candidate gene approaches can be used for detection of genetic potentials for cows, genetic improvement of cows will be accelerated much faster than the conventional breeding strategies which are sorely based on of phenotypic records.

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