Asian-Aust. J. Anim. Sci.
Vol. 21, No. 12 : 1689 - 1695
December 2008

www.ajas.info

SNP Discovery in the Leptin Promoter Gene and Association with Meat Quality and Carcass Traits in Korean Cattle

E. R. Chung*, S. C. Shin, K. H. Shin and K. Y. Chung

Division of Animal Science and Biotechnology, College of Life Science and Natural Resources, Sangji University, Wonju 220-702, Korea

ABSTRACT : Leptin, the hormone product of the obese gene, is secreted predominately from white adipose tissue and regulates feed intake, energy metabolism and body composition. It has been considered a candidate gene for performance, carcass and meat quality traits in beef cattle. The objective of this study was to identify SNPs in the promoter region of the leptin gene and to evaluate the possible association of the SNP genotypes with carcass and meat quality traits in Korean cattle. We identified a total of 25 SNPs in the promoter region (1,208-3,049 bp upstream from the transcription start site) of the leptin gene, eleven (g.1508C>G, g.1540G>A, g.1545G>A, g.1551C>T, g.1746T>G, g.1798ins(G), g.1932del(T), g.1933del(T), g.1934del(T), g.1993C>T and g.2033C>T) of which have not been reported previously. Their sequences were deposited in GenBank database with accession number DQ202319. Genotyping of the SNPs located at positions g.2418C>G and g.2423G>A within the promoter region was performed by direct sequencing and PCR-SSCP method to investigate the effects of SNP genotypes on carcass and meat quality traits in Korean cattle. The SNP and SSCP genotypes from the two mutations of the leptin promoter were shown to be associated with the BF trait. The average BF value of animals with heterozygous SNP genotype was significantly greater than that of animals with the homozygous SNP genotypes for the g.2418C>G and g.2423G>A SNPs ($p<0.05$). Analysis of the combined genotype effect in both SNPs showed that animals with the AC SSCP genotype had higher BF value than animals with BB or AA SSCP genotypes ($p<0.05$). These results suggest that SNP of the leptin promoter region may be useful markers for selection of economic traits in Korean cattle. (**Key Words :** Leptin Promoter, SNP Identification, Meat Quality, Korean Cattle)

INTRODUCTION

The challenge to the beef cattle industry in Korea is the production of cattle that exhibit satisfactory or superior meat quality. Marbling (intramuscular fat) is the most important meat quality trait in Korea because carcass value is primarily determined by the degree of marbling. In particular, eating quality traits such as taste, juiciness and tenderness of meat, are influenced by the amount of intramuscular fat (Platter et al., 2005).

Leptin is synthesized and expressed predominantly by white adipocytes. The expression and secretion of leptin is highly correlated with body fat mass and adipocyte size (Houseknecht et al., 1998). It affects regulation of food intake, energy partition and body composition (Houseknecht et al., 1998; Baile et al., 2000; Dai et al., 2007). Due to the essential role in physiological mechanisms related to adipocytes and fat deposition of mammals, the leptin gene has been considered as a strong candidate gene for its use as a genetic marker for carcass and meat quality traits (Fitzsimmons et al., 1998; Buchanan et al., 2002; Lagonigro et al., 2003; Schenkel et al., 2005).

Many studies have shown a close relationship between leptin gene polymorphisms and carcass and meat quality traits of beef cattle (Buchanan et al., 2002; Nkrumah et al., 2004; Schenkel et al., 2005). Although previous studies have focused on associations between polymorphisms in the coding regions of the leptin gene and economically important traits, studies in human and other species have shown that polymorphisms in the leptin promoter may be of major importance (Nkrumah et al., 2005). This is because such polymorphisms are generally associated with the sequence elements and factors regulating gene expression and may completely abolish the inducibility of the promoter (Miller et al., 1996) or decrease its activity significantly (Mason et al., 1998; Nkrumah et al., 2005). Associations of polymorphisms within exon region of the leptin gene with

* Corresponding Author: E. R. Chung. Tel: +82-33-730-0541, Fax: +82-33-730-0503, E-mail: erchung@sangji.ac.kr
Received February 11, 2008; Accepted July 8, 2008

Table 1. Primer sequences for sequencing and SNP screening in the promoter region of the bovine leptin gene

Primer sequence	Location	Fragment size (bp)	Annealing Temp. (°C)	GenBank accession no.
5'-TCCCAATCTGACCTCTGACC-3' 5'-AAACATCAGGGCGTTTCATC-3'	1208-2245	1,038	54	AB070368
5'-AGCAAAACAACCAGGCTCAAAC-3' 5'-AGGAGAGAGCCGGGCACTTA-3'	2050-3049	1,000	57	

carcass and meat quality traits recently were reported in Korean cattle, known as Hanwoo (Kong et al., 2006; Shin and Chung, 2007a). However, there have been no studies on the association between polymorphisms of the leptin promoter gene and carcass traits in Korean cattle. The present study was conducted to identify SNP in the promoter region of the leptin gene and to evaluate its possible associations with carcass and meat quality traits in Korean cattle.

MATERIALS AND METHODS

Animals and carcass data

The Korean native cattle genomic DNA samples were obtained from 275 steers produced from 52 sires used in progeny testing program of National Livestock Research Institute (NLRI) of Korea in 2004 and 2005. The animals were fed using standard feeding programs in the Daekwanryeong and Namwon branch stations. All animals were slaughtered at 25 months of age. Meat samples were collected from 13th thoracic rib to the first lumbar vertebrae of the steers within 24 h of slaughter and their meat quality was evaluated by mechanical and physical methods. The carcass data included were carcass weight (CW), carcass percentage (CP), M. *longissimus dorsi* area (LDA), backfat thickness (BF) and marbling score (MS). BF and LDA were measured at the 12th- and 13th- rib interface. MS for quality grade was evaluated on a cross section of the longissimus muscle at the 12^{th}-to 13^{th}-rib interface by official graders in accordance with the Korea Meat Grading Standards. MS is scored on a scale from 1 to 7 with 7 being associated with the most marbling.

Sequence analysis and SNP identification

Genomic DNA was extracted from white blood cells using a saturated salt procedure (Miller et al., 1988). Identification of SNPs in the bovine leptin promoter used the approximately 1.84-kb promoter region (1,208-3,049 bp) upstream from the transcription start site (GenBank accession no. AB070368). Two primer sets were designed to cover the entire leptin promoter region (Table 1), and pooled DNA samples from the sixty unrelated animals were amplified by PCR and directly sequenced in both directions using BigDye™ Terminator V3.1 Cycle Sequencing Kit in an ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Sequencer Software V5.1 (Applied Biosystems) was used to assemble the sequences and to identify polymorphisms.

SNP marker genotyping

Genotyping of the two SNPs located at positions g.2418C>G and g.2423G>A (numbering according to GenBank database accession no. AB070368) within the promoter region of the leptin gene was performed by direct sequencing, respectively. The combined effects of the two SNPs were also estimated. The combined genotypes of the two SNPs were analyzed using a PCR-SSCP method, because no restriction site exists for these SNPs. The PCR amplification was carried out using forward (5′-GTTA AACCTAAATTTGCGA -3′) and reverse (5′-ATCACACCT GCCTTGATGAT -3′) primers designed for amplification of a 252 bp fragment including two SNP sites, g.2418C>G and g.2423G>A. The 20 μl reaction mixture contained 30 ng of genomic DNA, 0.05 μM of each primer, 10×PCR buffer, 1.5 mM $MgCl_2$, 250 μM of each dNTP and 1.0 unit *Taq* polymerase. Amplification conditions were 94°C for 5min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 5 min in a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT). After PCR amplification, 2 μl of PCR product was mixed with 8 μl of gel loading solution containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol. The mixture was then denaturated at 96°C for 5 min, cooled on ice for 5 min and loaded on a nondenaturing 12% polyacrylamide gels (49:1 acrylamide to bis-acrylamide). Electrophoresis was performed in 1×TBE buffer at 250 V for 4 h at room temperature. After electrophoresis, the DNA fragments in the gel were detected by silver staining. The PCR products with different genotypes were sequenced to confirm the nucleotide change of the SNP.

Statistical analysis

The association of the SNP marker genotypes with carcass and meat quality traits were determined by analysis of variance of quantitative traits using GLM procedures of SAS (SAS, Inst. Inc., Cary NC, USA). Significant differences between least square means of the different genotypes were calculated using a LSMEANS contrast procedure. The SNP genotype effects on phenotypic value

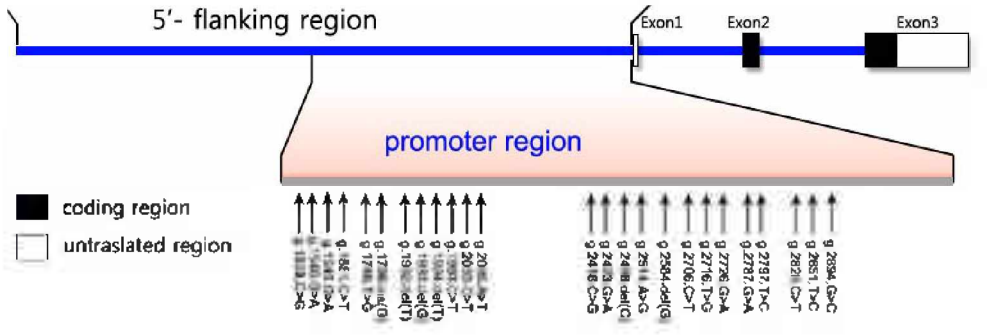

Figure 1. Map of SNPs identified in the promoter region of the leptin gene on bovine chromosome 4.

of each trait were evaluated by the following model:

$$Y_{ijklmn} = \mu + S_i + YS_j + P_k + T_l + A_m + G_n + e_{ijklmn}$$

Where Y_{ijklmn} is the observation of the carcass traits, μ is the overall mean for each trait, S_i is the effect of i_{th} sire, YS_j is the effect of j_{th} year and season of calving, P_k is the effect of the k_{th} parity, T_l is the effect of the l_{th} progeny testing station, A_m is the covariate for age of slaughter, G_n is the fixed effect of n_{th} SNP genotype for leptin promoter and e_{ijklmn} is the random residual effect.

Additive and nonadditive genetic effects were estimated for traits that were different ($p<0.05$) between animals with different SNP genotypes. Additive genetic effects were estimated by the difference between solutions for the two homozygous genotypes. Dominance deviation was estimated by the differences between the solution for the heterozygous genotype and the average of the solutions for the two homozygous genotypes.

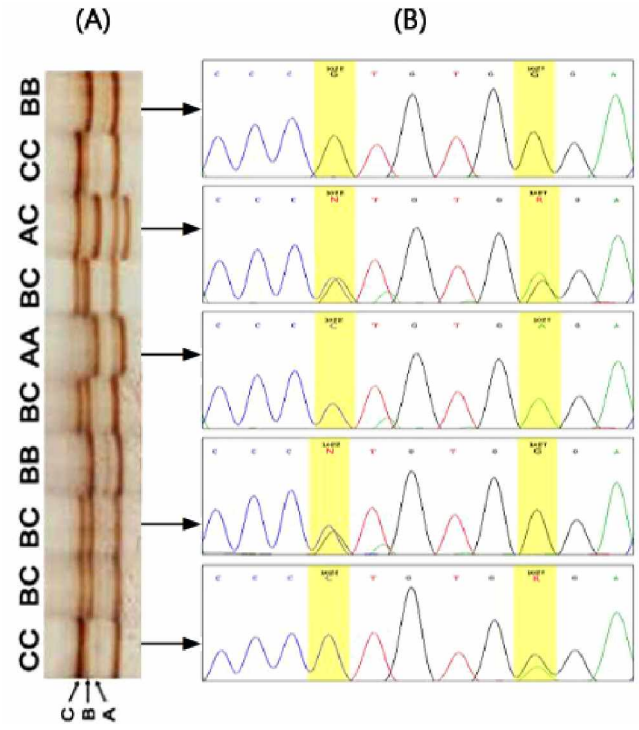

Figure 2. Polyacrylamide gel showing five different genotypes by the PCR-SSCP method (A) and sequence chromatograms for the SNP detection of the each SSCP genotype in promoter region of the leptin gene (B).

RESULTS

SNP identification and genotyping

DNA samples from sixty unrelated individuals were amplified and sequenced. The sequence information obtained by direct sequencing of the PCR fragments was used to detect SNPs in the promoter region of the leptin gene in Korean cattle. In this study, we identified a total of 25 polymorphic sites in leptin promoter region (Figure 1). Their sequences were deposited in GenBank database with accession number DQ202319. Among identified polymorphisms, two SNPs (g.2418C>G and g.2423G>A) were selected for association analysis by large-scale genotyping based on frequency. Observations of the genotypes for animals in the experimental population revealed that all animals that had genotypes CC, CG or GG for g.2418C>G also had genotypes GG, GA or AA for g.2423G>A, respectively. The PCR-SSCP method was developed successfully for the combined genotype of these two SNPs because of very close position (5 bp distance) between the two SNPs. Five of the six possible SSCP genotype from the two SNPs were observed and designated as AA, BB, CC, AC and BC, which were generated by combinations of g.2418C>G and g.2423G>A SNPs (Figure 2). The nucleotide sequence analyses of the five SSCP genotypes revealed different allele combinations at positions 2418 (C to G transition) and 2423 (G to A transition): C/C and A/A in AA genotype, G/G and G/G in BB genotype, C/C and G/A in CC genotype, C/G and G/A in AC genotype and C/G and G/G in B/C genotype. No individuals heterozygous AB were found in the present study. At the g.2418C>G SNP, allele frequencies of the C

Table 2. Least squares means and standard errors for carcass and meat quality traits of different SNP genotypes (g.2418C>G) in the promoter region of the leptin gene in Korean cattle

Traits	SNP genotype			p-value	Effect	
	CC	CG	GG		Additive	Dominance
LW (kg)	539.315±8.092	544.502±6.971	534.070±9.511	0.656	5.245±11.814	-15.619±18.592
CW (kg)	307.773±5.246	311.118±4.519	306.401±6.166	0.794	1.372±7.659	-8.061±12.054
DP (%)	56.997±0.257	57.088±0.222	57.339±0.302	0.650	-0.342±0.376	0.160±0.592
BF (cm)	0.616±0.038^{ab}	0.710±0.033^{a}	0.554±0.045^{b}	0.015*	-0.061±0.056	-0.249±0.088**
EMA (cm^2)	75.219±1.229	74.427±1.058	75.004±1.444	0.877	0.214±1.794	1.368±2.824
MS1 (1-7)	1.720±0.210	2.091±0.181	2.215±0.247	0.224	-0.495±0.307	-0.247±0.483

LWT = Live weight; CWT = Carcass weight; DP = Dressing percentage; BF = Backfat thickness.
LDA = M. *Longissimus dori* area; MS = Marbling score.
* Effect was significant at p<0.05. ** Effect was significant at p<0.01.
$^{a, b}$ Different superscript letters within a raw are significantly different (p<0.05).

and G were 0.548 and 0.452, respectively. For the g.2423G>A SNP, the frequencies of the G and A alleles were 0.633 and 0.367, respectively. The frequencies of SSCP genotype were 21.1% for AA, 23.1% for BB, 11.6% for CC, 19.7% for AC and 24.5% for BC.

Association of SNP genotypes with carcass traits

The association of the SNP genotypes in the two SNPs (g.2418C>G and g.2423G>A) of the leptin promoter region with the carcass and meat quality traits was examined using least square methods. There was a significant association between the two leptin promoter SNP genotypes and BF trait. At the position 2418 with C/G transition, animals with CG SNP genotype had higher BF compared with GG SNP genotype (p<0.015) (Table 2). At the position 2423 with G/A transition, animals with GA SNP genotype had higher BF compared with GG or AA SNP genotypes (p<0.006) (Table 3). Similar effects were also observed with the combined SSCP genotypes of the two SNPs (g.2418C>G and g.2423G>A) on BF trait. Animals with the SSCP genotype AC had higher BF than animals with BB or AA SSCP genotypes (p<0.007) (Table 4). Animals with the AC genotype were approximately 0.229 and 0.211 cm fatter than animals with the BB and AA genotypes, respectively.

Table 3. Least squares means and standard errors for carcass and meat quality traits of different SNP genotypes (g.2423G>A) in the promoter region of the leptin gene in Korean cattle

Traits	SNP genotype			p-value	Effect	
	GG	GA	AA		Additive	Dominance
LW (kg)	538.674±6.780	541.766±8.324	543.069±10.181	0.911	4.394±11.499	-1.789±21.152
CW (kg)	307.985±4.390	310.197±5.389	309.461±6.592	0.942	1.476±7.445	-2.949±13.695
DP (%)	57.125±0.215	57.215±0.264	56.906±0.323	0.758	-0.219±0.365	-0.399±0.672
BF (cm)	0.605±0.032^{b}	0.748±0.039^{a}	0.570±0.048^{b}	0.006**	-0.034±0.054	-0.322±0.099**
EMA (cm^2)	74.336±1.024	75.759±1.257	74.302±1.538	0.647	-0.033±1.737	-2.879±3.195
MS1 (1-7_	2.224±0.175	1.808±0.215	1.788±0.263	0.173	-0.435±0.297	0.396±0.547

LWT = Live weight; CWT = Carcass weight; DP = Dressing percentage; BF = Backfat thickness.
LDA = M. *Longissimus dori* area; MS = Marbling score.
** Effect was significant at p<0.01.
$^{a, b}$ Different superscript letters within a raw are significantly different (p<0.05).

Table 4. Least squares means and standard errors for carcass and meat quality traits of different SSCP genotypes (g.2418C>G and g.2423 G>A) in the promoter region of the leptin gene in Korean cattle

Traits	SNP genotype					p-value
	AA	AC	BB	BC	CC	
LW (kg)	542.965±10.224	547.192±10.580	534.314±9.582	542.789±9.179	531.939±14.168	0.855
CW (kg)	309.457±6.630	313.589±6.860	306.507±6.213	309.416±5.952	304.129±9.187	0.924
DP (%)	56.918±0.325	57.283±0.336	57.333±0.304	56.938±0.292	57.108±0.450	0.812
BF (cm)	0.568±0.047^{b}	0.779±0.049^{a}	0.550±0.044^{b}	0.655±0.042^{ab}	0.692±0.066^{ab}	0.007**
EMA (cm^2)	74.321±1.548	75.217±1.601	74.936±1.450	73.774±1.389	76.750±2.144	0.820
MS1 (1-7)	1.790±0.265	1.913±0.274	2.221±0.248	2.229±0.238	1.623±0.367	0.424

LWT = Live weight; CWT = Carcass weight; DP = Dressing percentage; BF = Backfat thickness.
LDA = M. *Longissimus dori* area; MS = Marbling score.
** Effect was significant at p<0.01.
$^{a, b}$ Different superscript letters within a raw are significantly different (p<0.05).

Consequently, the AC heterozygous SSCP genotype (CG SNP heterozygous genotype at position 2418 and GA SNP heterozygous genotype at position 2423) of the leptin promoter was found to have significant association with thicker BF value. The heterozygous genotype of each of the two SNPs for BF trait showed significant dominance effect. No significant association, however, was detected between any of the SSCP genotypes and other traits measured in this study.

DISCUSSION

Although the genes that affect a polygenic trait such as carcass and meat quality traits are unknown, a number of potential candidate genes have been identified and selected for association analyses on the basis of known relationship between physiological or biochemical processes and economically important traits (Yao et al., 1996). Leptin has been considered to play an important role in the regulation of appetite, energy partition and body composition in mammals (Schenkel et al., 2005; Choudhary et al., 2006; Yang et al., 2008). Therefore, the leptin has been suggested as an excellent candidate gene for genetic variation in carcass and meat quality traits in beef cattle (Buchanan et al., 2002; Schenkel et al., 2005; Shin and Chung et al., 2007a). In this study, the bovine leptin promoter was chosen as a candidate gene for association tests with carcass traits in Korean cattle. The leptin gene consists of three exons, separated by two introns with the coding regions in exons 2 and 3, and several SNPs have been previously reported in both introns and exons of the leptin gene among different breeds of cattle (Konfortov et al., 1999; Lagonigro et al., 2003). In Korean cattle, Yoon et al. (2005) identified fifty-seven SNPs in leptin gene: fourteen in 5′ flanking region, twenty-seven in introns, eight in exons and eight in 3′ flanking region. Among the polymorphisms identified, thirty-six SNPs were newly identified, and twenty-one SNPs, which were reported in other breeds, were also confirmed in Korean cattle. Additionally, three new SNPs (g.207C>T, g.528C>T and g.1759G>C) in the 5′ untranslated promoter region of the bovine leptin gene were reported in hybrid cattle (Nkrumah et al., 2005). In the present study, we found a total of twenty-five SNPs in the leptin promoter region and also confirmed fourteen SNPs reported previously in Korean cattle. However, eleven SNPs (g.1508C>G, g.1540G>A, g.1545G>A, g.1551C>T, g.1746T>G, g.1798ins(G), g.1932del(T), g.1933del(T), g.1934del(T), g.1993C>T and g.2033C>T) in the promoter region (1,208-3,049 bp upstream from the transcription start site) were newly identified in this study. The G to C substitution located at position 1759 in the promoter region reported by Nkrumah et al. (2005) was not observed in Korean cattle.

The leptin gene itself may be considered a potential QTL, influencing different production traits in cattle, for example, carcass and meat quality (Buchanan et al., 2002; Schenkel et al., 2005, Cheong et al., 2006; Kong et al., 2006; Shin and Chung, 2007a), milk production traits (Madeja et al., 2004), reproductive performance (Almeida et al., 2003) and feed intake (Lagonigro et al., 2003). Previous studies have identified SNPs in the coding regions of the leptin gene in cattle that show considerable associations with several production traits. In Korean cattle, Shin and Chung (2007a) reported that the g.1180C>T SNP in the exon 2 was associated with BF and MS. Animals with the genotype CC had higher BF than animals with TT genotype and higher MS compared with CT or TT genotypes, respectively. Kong et al. (2006) also reported that the association of two SNPs in the exon 2 region with carcass traits using ultrasound measurements. However, our results report association between SNP in the promoter region of the leptin gene and carcass traits in Korean cattle. The results showed that a specific SNP and SSCP genotype of the leptin promoter gene was significantly associated with BF trait. The heterozygous genotypes of the two SNPs (g.2418C>G and g.2423G>A) showed higher BF value compared with the homozygous genotypes, respectively. In the analysis of combined genotype effects of the two SNPs using a PCR-SSCP analysis, animals with the AC SSCP genotype showed also significantly higher BF value when compared with BB or AA SSCP genotypes. The dominance effect of each of the two SNPs was also significant for BF value. Thus, heterozygous animals were shown to have more desirable BF thickness. This may explain the phenomenon of heterosis (Shin and Chung, 2007b). The two SNPs are very close each other (only 5 bp distance) and the effect of the two SNPs closely linked in the promoter region on BF trait seems to be similar. Therefore, the significant linkage disequilibrium between the two SNPs may be existed and it suggests that the effect of one of the SNP may reflect an indirect effect of the other SNP (Nkrumah et al., 2005). Nkrumah et al. (2005) also reported associations between SNP in the 5′ flanking region of the bovine leptin gene with serum leptin concentration, growth, feed intake, feeding behavior and carcass merit in hybrid cattle. They showed that animals with the TT genotypes of a less frequent C/T substitution detected at position 528 show 48 and 39% increases in serum leptin concentration, 39 and 31% increases in backfat thickness, and 13 and 9% increases in marbling score, compared with CC or CT genotypes, respectively. Information of the possible mutations occurring at the promoter region of leptin gene can be useful to elucidate the regulatory mechanisms of the gene in body fat mass, fat deposition and adiposity of livestock. The promoter region of the leptin gene contains regulatory sequences which control the expression of leptin

and interact with a large number of *cis*-acting and *trans*-acting factors (Houseknecht et al., 1998). The leptin promoter have revealed functional binding sites for C/EBPα (CCAAT/enhancer binding protein α) in the region relative to the transcriptional site (Hwang et al., 1996). The C/EBPα functions as a transcriptional activator of certain adipocyte genes (Christy et al., 1989) and plays a role in terminal adipocyte differentiation (Umek et al., 1991). Transcriptional regulation of the leptin gene also seems to be controlled by PPARγ, which functions as trans-activators of fat-specific genes and dominant activators of fat cell differentiation (Tontonoz et al., 1994; Houseknecht et al., 1998). Although the exact molecular and physiological mechanisms underlying the association of the SNPs of the leptin promoter region with carcass traits reported in the present study are unknown, our results suggest that the promoter region of the leptin gene may be one of the candidate genes that control carcass traits or that the gene may be linked to some QTL that affect carcass traits in beef cattle. Association studies using more SNPs of the leptin promoter region and functional polymorphism of candidate genes may lead to the identification of causal mutations in the genes that control carcass and meat quality traits in beef cattle. Our results represent the initial associations of the SNPs in the leptin promoter region with carcass and meat quality traits in Korean cattle. Although further analysis are required to confirm our results, the association of SNP genotype in the leptin promoter region with better carcass traits is a very interesting finding and could be used in marker assisted selection to improve the carcass quality traits such as BF thickness in Korean cattle.

ACKNOWLEDGMENTS

This research was supported by Sangji University Research Fund, 2007.

REFERENCES

Almeida, S. E. M., E. A. Almedia, J. C. F. Moraes and T. A. Weimer. 2003. Molecular marker in the LEP gene and reproductive performance of beef cattle. J. Anim. Breed. Genet. 120:106-113.

Baile, C. A., M. A. Della-Fera and R. J. Martin. 2000. Regulation of metabolism and body fat mass by leptin. Annu. Rev. Nutr. 20:105-127.

Buchanan, F. C., C. J. Fitzsimmutz, A. G. Van Kessel, T. D. Thue, D. C. W. Sim and S. M. Schmutz. 2002. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genet. Sel. Evol. 34:105-116.

Cheong, H. S., D. H. Yoon, L. H. Kim, B. L. Park, E. R. Chung, H. J. Lee, I. C. Cheong, S. J. Oh and H. D. Shin. 2006. Leptin polymorphisms associated with carcass traits of meat in Korean cattle. Asian-Aust. J. Anim. Sci. 19:1529-1535.

Christy, R. J., V. W. Yang, J. M. Ntambi, D. E. Geiman, W. M. Landschulz, A. D. Friedman, Y. Nakabeppu, T. J. Kelly and M. D. Lane. 1989. Differentiation – induced gene expression in 3T3-L1 preadipocytes: CCAAT/enhancer binding protein interacts with and activates the promoter of two adipocyte-specific gene. Genes Dev. 3:1323-1335.

Choudhary, V., P. Kumar, V. K. Saxena, T. K. Bhattacharya, B. Bhushan, A. Sharma and K. A. Ahmed. 2006. Effect of leptin and IGFBP-3 gene polymorphisms on serum IgG level of cattle calves. Asian-Aust. J. Anim. Sci. 19:1095-1099.

Dai, H. C., L. Q. Long, X. W. Zhang, W. M. Zhang and X. X. Wu. 2007. Cloning and expression of the Duck leptin gene and the effect of leptin on food intake and fatty deposition in mice. Asian-Aust. J. Anim. Sci. 20:850-855.

Fitzsimmons, C. J., S. M. Schmutz, R. D. Bergen and J. J. Mckinnon. 1998. A potential association between the BM1500 microsatellite and fat deposition in beef cattle. Mamm. Genome 9:432-434.

Houseknecht, K. L., C. A. Baile, R. L. Matteri and M. E. Spurlock. 1998. The biology of leptin: A review. J. Anim. Sci. 76:1405-1420.

Hwang, C., S. Mandrup, O. A. MacDougald, D. E. Geiman and M. D. Lane. 1996. Transcriptional activation of the mouse obese(ob) gene by CCAAT/enhncer binding protein α. Proc. Natl. Acad. Sci. USA. 93:873-877.

Kong, H. S., J. D. Oh, S. G. Lee, Y. S. Hong, W. I. Song, S. J. Lee, H. C. Kim, B. H. Yoo, H. K. Lee and G. J. Jeon. 2006. Association of polymorphisms in the bovine leptin gene with ultrasound measurements for improving in Korea cattle. Asian-Aust. J. Anim. Sci. 19:1691-1695.

Konfortov, B. A., V. E. Licence and J. R. Miller. 1999. Resequencing DNA from a diverse panel of cattle reveals a high level of polymorphism in both intron and exon. Mamm. Genome. 10:1142-1145.

Lagonigro, R., P. Wiener, F. Pilla, J. A. Woolliams and J. L. Williams. 2003. A new mutation in the coding region of the bovine leptin gene associated with feed intake. Anim. Genet. 34:371-374.

Mason, M. M., Y. He, H. Chen, M. J. Quon and M. Reitman. 1998. Regulation of leptin promoter function by SP1, C/EBP, and a novel factor. Endocrinol. 139:1013-1022.

Madeja, Z., T. Adamowicz, A. Chmurzynska, T. jankowski, J. Melonek, M. Switonski and T. Strabel. 2004. Effect of leptin gene polymorphisms on breeding value for milk production traits. J. Dairy Sci. 87:3925-3927.

Miller, S. G., P. De Vos, M. Guerre-Millo, K. Wong, T. Hermann, B. Staels, M. R. Briggs and J. Auwerx. 1996. The adipocytes specific transcription factor C/EBPα modulates human ob gene expression. Proc. Natl. Acad. Sci. 93:5507-5511.

Miller, S. A., D. D. Dykes and H. F. Polesky. 1998. A simple salting out procedure for extracting DNA from human uncleated cells. Nucleic Acids Res. 16:1215.

Nkrumah, J. D., C. Li, J. A. Basarab, S. Guercio, Y. Meng, B. Murdoch, C. Hansen and S. S. Moore. 2004a. Association of a single nucleotide polymorphism in the bovine leptin gene with feed intake, growth, feed efficiency, feeding behavior and carcass merit. Can. J. Anim. Sci. 84:211-219.

Nkrumah, J. D., C. Li, J. Yu, C. Hansen, D. H. Keisler and S. S.

Moore. 2005. Polymorphism in the bovine leptin gene promoter associated with serum leptin concentration, growth, feed intake, feeding behavior, and measures of carcass merit. J. Anim. Sci. 83:20-28.

Platter, W. J., J. D. Taume, K. E. Belk, S. R. Koontz, P. L. Chapman and G. C. Smith. 2005. Effect of marbling and shear force on consumers` willingness to pay for beef strip loin steaks. J. Anim. Sci. 80:2825-2832.

Schenkel, F. S., S. P. Miller, X. Ye, S. S. More, J. D. Nkrumah, C. Li, J. Yu, I. B. Mandell, J. W. Wilton and J. L. Williams. 2005. Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. J. Anim. Sci. 83:2009-2020.

Shin, S. C and E. R. Chung. 2007a. Association of SNP marker in the leptin gene with carcass and meat quality traits in Korean cattle. Asian-Aust. J. Anim. Sci. 20:1-6.

Shin, S. C. and E. R. Chung. 2007b. SNP detection of carboxypeptidase E gene and its association with meat quality and carcass traits in Korea cattle. Asian-Aust. J. Anim. Sci. 20:328-333.

Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari and B. M. Spiegelman. 1994. mPPARγ2: Tissue-specific regulator of an adipocyte enhancer. Genes Dev. 8:1224-1234.

Umek, R. M., A. D. Friedman and S. L. Mcknight. 1991. CCAAT/ enhancer binding protein : A component of a differentiation switch. Sci. (Wash DC) 251:288-292.

Yang, S. H., H. Kawachi, M. A. Khan, S. Y. Lee, H. S. Kim, J. K. Ha, W. S. Lee, H. J. Lee, K. S. Ki, S. B. Kim, S. Sakaguchi, S. Maruyama and H. Yano. 2008. Development of bovine specific leptin radioimmunoassay and relationship of plasma leptin with vitamin A and age of Wagyu. Asian-Aust. J. Anim. Sci. 21:1286-1295.

Yao, J., S. E. Aggrey, D. Zadworny, J. F. Hayes and U. Kühnlein. 1996. Sequence variations in the bovine growth hormone gene characterized by single-strand conformation polymorphism (SSCP) analysis and their association with milk production traits in Holsteins. J. Anim. Sci. 144-1809-1816.

Yoon, D. H., B. H. Cho, B. L. Park, Y. H. Choi, H. S. Cheong, H. K. Lee, E. R. Chung, I. C. Cheong and H. D. Shin. 2005. Highly polymorphic bovine leptin gene. Asian-Aust. J. Anim. Sci. 18:1548-1551.