

## Association between Microsatellite DNA Marker of Leptin Gene and Carcass Traits in Korean Cattle

Eui-Ryong Chung\* and Ku-Young Chung<sup>1</sup>

Department of Biotechnology, Sangji University

<sup>1</sup>Department of Animal Science and Technology, Sangji University

### Abstract

Leptin, the product of the obesity (*ob*) gene, is synthesized in adipocytes or fat cells and has been implicated in the regulation of food intake, energy balance and body composition in mammals. Therefore, the leptin gene could be a candidate gene controlling fat deposition, meat quality and carcass traits in cattle. In this study the microsatellite genotypes for leptin gene were determined and their effects on carcass traits and meat quality were estimated in Korean cattle. Six different microsatellite alleles within leptin gene were identified and gene frequencies of 173, 177, 184, 186, 190 and 192 bp alleles were 0.012, 0.308, 0.067, 0.260, 0.342 and 0.016, respectively. The microsatellite marker of the leptin gene showed a significant association with the carcass percentage (CP) and marbling score (MS). Animals with genotypes 192/192 and 177/184 had higher CP than animals with other genotypes. Animals with genotypes 184/192 and 177/184 had higher MS compared with animals with other genotypes. Thus, the results suggest that the 177, 184 and 192 bp alleles may be associated with increased carcass percentage and intramuscular fat levels. No associations were found between the microsatellite genotypes of the leptin gene and other carcass traits such as carcass weight (CW), backfat thickness (BF) and *M. longissimus dorsi* area (LDA). In conclusion, the microsatellite markers of the leptin gene may be useful for marker-assisted selection of carcass traits and meat quality in Korean cattle.

**Key words :** leptin, *ob* gene, microsatellite marker, carcass traits, Korean cattle

### Introduction

Meat quality and carcass composition are the most economically important traits in beef cattle production. In Korea, carcass value is determined on the basis of meat quality, especially, degree of marbling. Marbling (intramuscular fat) contributes to the palatability of beef since increasing amounts of marbling increase the juiciness and flavor of the meat. Currently the beef market for well-marbled beef is larger than the supply. Thus, the challenge to the Korean beef industry is the production of cattle that exhibit satisfactory or superior marbling in the appropriate cuts of meat, without filling other fat depots. However, genetic improvement of meat quality traits by selective breeding is difficult and expensive because

of the difficulties in collecting the trait data. A good strategy to improve meat quality and composition in beef cattle is the search of molecular markers in or around genes involved directly or indirectly in meat quality and carcass traits. The development of a molecular marker that would identify beef cattle with the genetic potential to deposit intramuscular fat would be a valuable tool to produce high quality meat. Such DNA-based markers could be used in marker-assisted selection (MAS) protocols to make more rapid progress for selecting these cattle types. Beef producers could use information derived from a DNA marker for meat quality and carcass traits along with the many other criteria used to select breeding stock.

Information on the biology of individual genes and gene function including information from other species provides the basis on which to investigate genes for their effects on traits of interest (Grindflek et al., 2002). This strategy has been termed the candidate gene approach (Rothschild and Soller,

\* Corresponding author : Eui-Ryong Chung, Department of Biotechnology, Sangji University, Wonju 220-702, Korea. Tel: 82-33-730-0541, Fax: 82-33-730-0503, E-mail: erchung@mail.sangji.ac.kr

1997). A large number of DNA-based markers in livestock have been discovered to date, but relatively little is known about which markers could be useful in evaluation of specific traits. Leptin is a 16-KDa protein produced by the obesity (*ob*) gene (Friedman and Halaas, 1998). Leptin is synthesized in adipocytes or fat cells and has been implicated in the control of food intake and energy composition in mammals (Geary et al., 2003). Leptin is perhaps linked to meat quality determinants such as marbling and is also involved in the regulation of body weight (Hossner, 1998). Therefore, the leptin gene could be a potential candidate gene controlling some proportion of fat deposition, meat quality and carcass traits in cattle. If the DNA marker of the leptin gene is associated with production traits of interest, then a DNA-based diagnostic test could be a potentially useful tool for genetic evaluation. Microsatellites, also known as short tandem repeats (STR), are ideal genetic markers due to the high degree of polymorphism and can be analyzed easily and reproducibly by the PCR and gel electrophoresis (Hirano et al., 1996). These markers vary in the number of repeated units, and most are (GT/CA)<sub>n</sub> dinucleotide repeats, which are abundantly distributed throughout the mammalian genome.

The objective of the present study was to identify microsatellite polymorphisms of the leptin gene and to investigate the relationship between microsatellite marker and carcass traits including meat quality in Korean cattle.

## Materials and Methods

### Animals and Carcass Data

Data obtained from 267 Hanwoo steers in a progeny-testing program at National Livestock Research Institute were used in this study. Meat samples were collected from 13th rib to the first lumbar vertebrae of the steers within 24hr of slaughter

and 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 was measured at the 12th and 13th vertebrae. Marbling is scored on a scale from 1 to 7 with 7 being associated with the most marbling.

### Microsatellite Marker Typing of Leptin Gene

Genomic DNA was extracted from whole blood using a salting out procedure by Miller et al. (1998). Based on the published nucleotide sequence information of the bovine leptin gene (Tellam, 1996), pairs of oligonucleotide primers were synthesized to amplify the region containing repetitive sequences, corresponding to nucleotides 247~282 in the deposited sequence as shown in Fig. 1. The forward and reverse primer sequences were *ob*-F 5'-TTGTAATCCTGCAATATCTTGTCC-3' and *ob*-R 5'-TAAACAGGCCGTAGCATACAG-3', respectively. PCR was performed in a reaction volume of 20µL using 50 ng of genomic DNA, 0.1 µM of each primer, 1 X PCR buffer, 1.5 mM MgCl<sub>2</sub>, 200 µM of each dNTP and 1.0 unit Taq polymerase. Amplification conditions were 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 5 min. After the PCR was completed, the PCR products were separated in a 6% polyacrylamide gel. After electrophoresis, the DNA bands in the gel were detected by silver staining (Bassam et al., 1991). Allele sizes were determined using an M13 standard sequencing ladder.

### Statistical Analysis

The PROC GLM procedure of SAS (SAS, Inst. Inc., Cary NC) was used to test the association between microsatellite marker genotypes of the leptin gene and carcass traits and meat quality. The liner model used as follows:

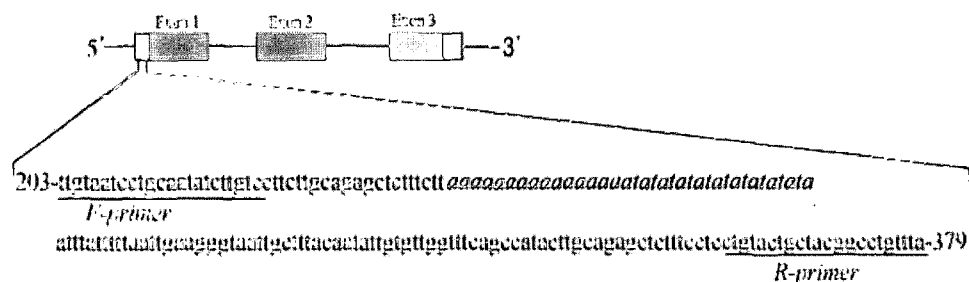


Fig. 1. Gene map of leptin on Chr 4 and partial sequences of the 5' UTR region of the gene. Coding exons are marker by black blocks and 5' and 3' UTR by white blocks.

$$Y_{ijk} = \mu + YS_i + LEP_j + e_{ijk}$$

Where  $Y_{ijk}$  is the observation of the carcass traits (CW, CP, LDA, BF and MS);  $\mu$  is the overall mean for each trait;  $YS_i$  is the effect of  $i$ th year and season of calving;  $LEP_j$  is the  $j$ th leptin genotype ( $j=AA, AB$  and  $BB$ ) and  $e_{ijk}$  is the random residual effect.

## Results and Discussion

Leptin, a 16-KDa protein secreted from white adipocytes, is believed to be involved in the regulation and deposition of fat (Wilkins and Davey, 1997). It has been suggested that leptin also plays a role in appetite regulation and weight control. As such it may play an important role in beef cattle and the availability of a highly polymorphic marker within the bovine leptin gene will facilitate genetic analysis to determine the specific roles in cattle. The bovine leptin gene has been mapped to chromosome 4 (Pomp et al., 1997; Stone et al., 1996) and its full sequence is available in GenBank (Tellam, R. L., accession number U50365). Many studies have suggested that leptin as a indicator of carcass composition in beef cattle is a major candidate gene for predicting carcass quality and carcass traits in beef cattle.

In this study, an experimental Korean cattle population was screened to identify microsatellite markers of the leptin gene and to evaluate their association with carcass traits and meat quality. The PCR products of leptin gene were separated 6% polyacrylamide gel and visualized by silver staining. The DNA band patterns of microsatellite in leptin gene are illustrated in Fig. 2. Six different alleles were identified in

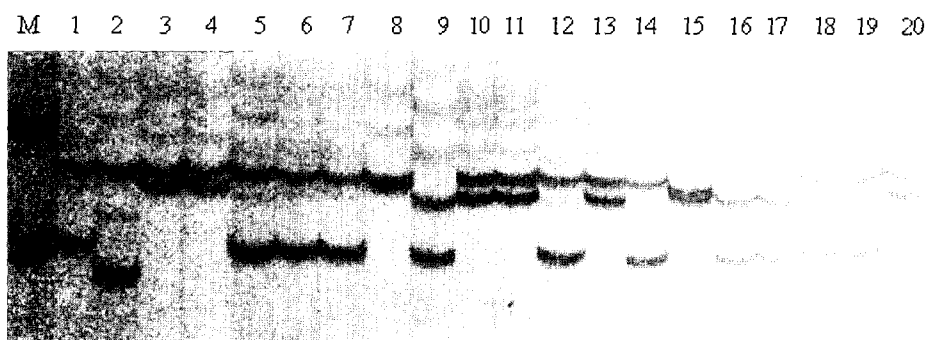
Korean cattle and their lengths were approximately 173, 177, 184, 186, 190 and 192 bp. However, the 209 bp allele was not found in the Korean cattle. The sequence of the bovine leptin gene revealed the putative microsatellite 5'-AAAAAA AAAAAAAAAAATATATATATATATATATA-3' in the 5' UTR region of the gene. This fragment corresponds to nucleotide 247 to 282 in the deposited sequence (Tellam, 1996). This region of the gene is surrounded by repetitive sequences and also a 16bp direct repeat (bases 229~246 and 339~354). Wilkins and Davey (1997) found 18 alleles in Holstein and Jersey dairy cattle, and Tessanne et al. (1998) observed five alleles (177, 184, 186, 190 and 209 bp) in Angus bulls. The microsatellite within the leptin gene proved to be highly polymorphic markers in cattle.

Allele and genotype frequencies of the microsatellite markers of leptin gene in Korean cattle are presented in Table 1. Frequencies of the 173, 177, 184, 186, 190 and 192 alleles

**Table 1. Genotype and allele frequencies of microsatellite marker of leptin gene in Korean cattle**

Genotype	Genotype frequencies	Allele	Frequencies
177/177	0.030	173	0.012±0.01 <sup>1)</sup>
177/184	0.049	177	0.308±0.02
177/186	0.198	184	0.067±0.01
177/192	0.333	186	0.260±0.02
184/186	0.026	190	0.016±0.01
184/192	0.060	192	0.342±0.02
186/186	0.022		
186/192	0.251		
192/192	0.030		

<sup>1)</sup> Standard error.



**Fig. 2. DNA banding pattern of microsatellite marker genotype in leptin gene.** Lanes 1, 5, 6, 7, 12, 14, 18 and 19, 192/172 types; lane 2, 192/173 type; lanes 3, 4, 10, 11, 13 and 20, 192/186 types; lane 8, 192/192 type; lane 9, 184/177 type; lane 15, 190/186 type; lanes 16 and 17, 186/177 types. M: size marker.

were 0.012, 0.308, 0.067, 0.260, 0.342 and 0.016, respectively. The genotype frequencies observed were 0.030 for the 177/177, 0.049 for the 177/184, 0.198 for the 177/186, 0.333 for the 177/192, 0.026 for the 184/186, 0.060 for the 184/192, 0.022 for the 186/186, 0.251 for the 186/192 and 0.030 for the 192/192 genotypes. The allele frequency was highest for allele 192 bp followed by alleles 177 bp and 186 bp, while the frequency of allele 184 bp was low and those of alleles 173 bp and 190 bp were extremely rare. Tessanne et al. (1998) reported that the frequencies of alleles 177, 184, 186, 190, 192 and 209 bp in Angus beef cattle were 0.57, 0.17, 0.04, 0.03, 0.17 and 0.03, respectively. Therefore, the allele frequencies of microsatellite marker in leptin gene were different between cattle breeds. Another microsatellite and several point mutations have also been detected in the bovine leptin gene. A microsatellite of the composition 5' GATA (CA)<sub>n</sub> CTAG 3' has been detected in the DNA flanking the 3' end of the obesity gene (Stone et al., 1996). This microsatellite has at least four alleles (Fitzsimmons et al., 1998).

Among the nine genotypes, the genotype 177/192 was the most frequent (33.3%) and the frequencies of the 186/192 and 188/186 genotypes were 25.1% and 19.8%, respectively. However, all the other genotypes were present in less than 10%. The leptin, the obese gene product, is a hormone in the fat metabolism pathway that has been shown to affect the amount of marbling in beef. Therefore, variations in it may make significant contributions to individual differences in growth and carcass characteristics of cattle.

Least squares means and standard errors of CW, CP, LDA,

BF and MS for different microsatellite marker genotypes of leptin gene are presented in Table 2. The microsatellite marker showed significant effects ( $p < 0.05$ ) on the CP and MS. The CP of 192/192 genotype ( $58.13 \pm 0.27$ ) and 177/184 genotype ( $57.78 \pm 0.43$ ) showed higher than those of other genotypes ( $56.34 \pm 0.58 \sim 56.77 \pm 0.39$ ). The MS of 184/192 genotype ( $3.21 \pm 0.62$ ) and 177/184 genotype ( $3.10 \pm 0.54$ ) showed higher than those of other genotypes ( $1.92 \pm 0.21 \sim 2.75 \pm 0.36$ ). No associations were found between the microsatellite genotypes of the leptin gene and other carcass traits such as CW, BF and LDA. The 177, 184 and 192bp alleles were associated with increased carcass weight and intramuscular fat levels. Thus, these microsatellite alleles and genotypes could be used as DNA marker for individual selection on carcass percentage and marbling score in Korean cattle. Fitzsimmons et al. (1998) found an association between alleles at the BM 1500 microsatellite (which maps close to the leptin gene) and the carcass traits % rib fat, % rib lean, average fat and grade fat in 158 purebred beef bulls. They reported that the presence of the 138bp allele in the genotype of an animal is correlated with higher levels of fat, whereas the 147bp allele has the opposite effect. Also, in another investigation by Tessanne et al. (1998), a significant relationship between leptin genotype and carcass traits was found. The result showed that microsatellite marker had a significant effect ( $p < 0.05$ ) on rib eye area in Angus cattle. Several studies have indicated an association between leptin levels and livestock production traits: serum leptin level has been positively correlated with rib eye fat thickness in beef cattle (Minton et al., 1998), while

**Table 2. Least square means and standard error of carcass traits by microsatellite marker genotype of leptin gene in Korean cattle**

Microsatellite genotype	Carcass traits				
	Carcass weight (kg)	Carcass percentage (%)	Backfat thickness (cm)	<i>M. longissimus dorsi</i> area (cm <sup>2</sup> )	Marbling score (1-7)
177/177	307.73 $\pm$ 7.36 <sup>1)</sup>	56.77 $\pm$ 0.39 <sup>a</sup>	0.77 $\pm$ 0.07	74.33 $\pm$ 0.48	1.92 $\pm$ 0.21 <sup>a</sup>
177/184	303.67 $\pm$ 4.30	57.78 $\pm$ 0.43 <sup>b</sup>	0.78 $\pm$ 0.03	74.86 $\pm$ 0.87	3.10 $\pm$ 0.54 <sup>b</sup>
177/186	308.25 $\pm$ 7.19	56.62 $\pm$ 0.62 <sup>a</sup>	0.73 $\pm$ 0.04	74.34 $\pm$ 1.23	2.64 $\pm$ 0.23 <sup>a</sup>
177/192	306.18 $\pm$ 3.23	56.34 $\pm$ 0.58 <sup>a</sup>	0.71 $\pm$ 0.04	74.62 $\pm$ 0.93	2.40 $\pm$ 0.28 <sup>a</sup>
184/186	302.42 $\pm$ 3.62	56.71 $\pm$ 0.46 <sup>a</sup>	0.74 $\pm$ 0.03	75.01 $\pm$ 0.62	2.75 $\pm$ 0.36 <sup>ab</sup>
184/192	310.56 $\pm$ 8.35	56.52 $\pm$ 0.31 <sup>a</sup>	0.73 $\pm$ 0.02	75.32 $\pm$ 0.83	3.21 $\pm$ 0.62 <sup>b</sup>
186/186	300.53 $\pm$ 8.38	56.46 $\pm$ 0.41 <sup>a</sup>	0.79 $\pm$ 0.09	74.12 $\pm$ 0.97	2.31 $\pm$ 0.26 <sup>a</sup>
186/192	308.14 $\pm$ 6.27	56.48 $\pm$ 0.26 <sup>a</sup>	0.75 $\pm$ 0.08	74.96 $\pm$ 0.74	1.98 $\pm$ 0.37 <sup>a</sup>
192/192	309.24 $\pm$ 6.29	58.13 $\pm$ 0.27 <sup>b</sup>	0.80 $\pm$ 0.06	74.57 $\pm$ 0.56	2.26 $\pm$ 0.18 <sup>a</sup>

<sup>a,b</sup> means with different superscripts in the same column are significantly different ( $p < 0.05$ ).

<sup>1)</sup> LSM $\pm$ SE.

administration of leptin reduces feed intake in sheep (Tokuda et al., 2000). Two polymorphisms in the porcine leptin gene have been tentatively associated with feed intake and growth trait (Kennes et al., 2001). Jiang and Gibson (1999) also reported a possible association between the leptin gene polymorphism and fatness in pigs. In human being and mice the expression and secretion of leptin is highly correlated with adipose tissue mass and declines after weight loss (Maffei et al., 1995). The ob gene is highly conserved among mammals, and the bovine and porcine leptin genes share over 89% similarity with their human counterparts. A better understanding of energy regulation and adipose tissue development will help in the treatment of human obesity and this knowledge may also prove important for the manipulation of meat production in livestock. Important economic factors for animal production, which may be influenced by leptin include feed conversion efficiency and intra-muscular fat, which is considered to improve meat quality (Wheeler et al., 1994; Savell and Cross, 1998).

In conclusion, we suggest that the microsatellite genotypes of the leptin gene as a candidate gene for fat characteristics may be useful for marker-assisted selection of carcass quality in Korean cattle. However, further study in broader populations will be necessary to confirm the associations observed in this study.

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### References

1. Bassam, J. B., Caetano-Anolles, G., and Gresshof, P. M. (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.* **196**, 80-83.
2. Fitzsimmons, C. J., Schmutz, S. M., Bergen, R. D., and McKinnon, J. J. (1998) A potential association between the BM 1500 microsatellite and fat deposition in beef cattle. *Mamm. Genome* **9**, 432-434.
3. Friedman, J. M. and Halaas, J. L. (1998) Leptin and the regulation of body weight in mammals. *Nature* **395**, 763-770.
4. Geary, T. W., McFadin, E. L., MacNeil, M. D., Grings, E. E., Short, R. E., Funston, R. N., and Keisler, D. H. (2003) Leptin as a predictor of carcass composition in beef cattle. *J. Anim. Sci.* **81**, 1-8.
5. Grindflek, E., Holzbauer, R., Plastow, G., and Rothschild, M. F. (2002) Mapping and investigation of the porcine major insulin sensitive glucose transport (SLC2A4/ GLUT4) gene as a candidate gene for meat quality and carcass traits. *J. Anim. Breed. Genet.* **119**, 65-68.
6. Hirano, J., Nakane, S., Mizoshita, K., Yamakuchi, H., Inoue-Murayama, M., Watanabe, T., Barendse, W., and Sugimoto, Y. (1996) Characterization of 42 highly polymorphic bovine microsatellite markers. *Anim. Genet.* **27**, 365-368.
7. Hossner, K. L. (1998) Cellular, molecular and physiological aspects of leptin: potential application in animal production. *Canadian J. Anim. Sci.* **78**, 463-472.
8. Jiang, Z. H. and Gibson, J. P. (1999) Genetic polymorphisms in the leptin gene and their association with fatness in four pig breeds. *Mamm. Genome* **10**, 191-193.
9. Kennes, Y. M., Murphy, B. D., Pothier, F., and Palin, M. F. (2001) Characterization of swine leptin (LEP) polymorphisms and their association with production traits. *Anim. Genet.* **32**, 215-218.
10. Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., and Fridman, J. M. (1995) Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Medicine* **1**, 1155-1161.
11. Miller, S. A., Dykes, D. D., and Polesky, H. F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 1215-1220.
12. Minton, J. E., Bindel, D. J., Drouillard, J. S., Titgemeyer, E. C., Grieger, D. M., and Hill, C. M. (1998) Serum leptin is associated with carcass traits in finishing cattle. *J. Anim. Sci. (Suppl.)* **76**, 231-237.
13. Pomp, D., Zou, T., Clutter, A. C., and Barendse, W. (1997) Rapid communication: Mapping of leptin to bovine chromosome 4 by linkage analysis of a PCR-based polymorphism. *J. Anim. Sci.* **75**, 1427-1433.
14. Rothschild, M. F. and Soller, M. (1997) Candidate gene analysis to detect genes controlling traits of economic importance in domestic livestock. *Probe* **8**, 13-20.
15. Savell, J. W. and Cross, H. R. (1998) The role of fat in the palatability of beef, pork and lamb. In: Designing

- foods, animal product options in the marketplace 99, 345-355.
16. Stone, R. T., Kappes, S. M., and Beattie, C. W. (1996) Two polymorphic microsatellites within an 18Kb genomic clone containing the bovine *ob* gene. *Anim. Genet.* 27, Suppl. 2, 64.
17. Tellam, R. L. (1996) *Bos taurus* leptin (obese) gene, complete CDs, GenBank Accession No. U50365.
18. Tessanne, K., Hines, H. C., and Davis, M. E. (1998) Relationships of polymorphisms in the bovine leptin gene with differences in beef carcass traits. Research and Reviews; Beef and Sheep, special circular 170-199.
19. Tokuda, T., Matsui, T., Ito, J., Torill, S., and Yano, H. (2000) The changes in body weight and plasma metabolic levels during leptin injection are caused by the reduction of food intake in sheep. *Animal Sci.* 70, 343-348.
20. Wheeler, T. L., Cundift, L. V., and Koch, R. M. (1994) Effect of marbling degree on beef palatability in *Bos taurus* and *Bos indicus* cattle. *J. Anim. Sci.* 72, 3145-3151.
21. Wilkins, R. J. and Davey, H. W. (1997) A polymorphic microsatellite in the bovine leptin gene. *Anim. Genet.* 28, 376-383.
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