Leptin Polymorphisms Associated with Carcass Traits of Meat in Korean Cattle

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ABSTRACT: Leptin has been investigated as a candidate gene for fat characteristics in beef cattle. Previously, we have reported 57 sequence variants discovered in Korean cattle (Bos Taurus coreanae). In this study, we examined the association between polymorphisms of leptin and carcass traits (cold carcass weight (CWT) and marbling score (Marb)) in Korean cattle. Among 57 polymorphisms, 11 common polymorphic sites were genotyped in our beef cattle (n = 437). Statistical analysis revealed that one single nucleotide polymorphism in coding exon (c.+411T>C (A137A)) showed a significant association with the yield trait, CWT. The C-bearing genotypes (CC or CT) of c.+411T>C (A137A) showed the higher CWT (p = 0.006). c.+150C>G (S50S) also showed a significant association with the quality trait, Marb (p = 0.01). Our findings suggest that polymorphisms in leptin might be one of the important genetic factors that influence carcass yield and quality in beef cattle, especially in CWT and Marb. (Key Words: Leptin, Cold Carcass Weight, Marbling Score, Polymorphism)

INTRODUCTION

Leptin is a protein hormone with important effects in regulating body weight, metabolism and reproductive function (Santos-Alvarez et al., 1999; Kadokawa et al., 2000; Block et al., 2001). The protein is approximately ~16 kDa in mass and encoded by the obese gene, leptin (Halaas et al., 1995). Leptin is expressed predominantly by adipocytes, which fits with the idea that body weight is sensed as the total mass of fat in the body (Block et al., 2001). Small amounts of leptin are also secreted by cells in the epithelium of the stomach and in the placenta (Reseland et al., 1999). Leptin receptors are highly expressed in areas of the hypothalamus known to be important in regulating body weight, as well as in T lymphocytes and vascular endothelial cells (Clement et al., 1998).

Genetically obese mice with inactivating mutations in the leptin or the gene encoding the leptin receptor have been known for many years (Halaas et al., 1995). Recent studies with obese and non-obese humans demonstrated a strong positive correlation of serum leptin concentrations with percentage of body fat, and also that there was a higher concentration of leptin mRNA in fat from obese compared to thin subjects (Considine, 1997; Montague et al., 1997; Friedman, 1998). It also appears that as adipocytes increase in size due to accumulation of triglyceride, they synthesize more leptin (Soukas et al., 2000). The effects of leptin on body weight are mediated through effects on hypothalamic centers that control feeding behavior, hunger, body temperature and energy expenditure (Jequier, 2002; Palkovits, 2003). Daily injections of recombinant mouse or human leptin into ob/ob mice led to a dramatic reduction in food intake within a few days, and to roughly a 50% reduction in body weight within a month (Pelleymouenter et al., 1995; Halaas et al., 1997).

Since the bovine leptin has been identified on chromosome 4 (Stone et al., 1996), several studies have revealed that polymorphisms in leptin were associated with lean and fat cattle (Fitzsimmons et al., 1998) and with the fat contents and feed intakes (Konfortov et al., 1999; Buchanan et al., 2002; Buchanan et al., 2003; Lagonigro et al., 2003). This genetic information regarding fatty-phenotypic traits is valuable for breeding for high quality meat through breeding by marker-assisted selection (MAS). Important economic factors for animal production that may be influenced by leptin include feed conversion efficiency and intra-muscular fat, which is considered to improve meat quality and quantity (Wheeler et al., 1994; Baik et al., 2002; Chung et al., 2005).
Table 1. Means and standard variations (SD) for APGS carcass grade traits in Korean male beef cattle (n = 437)

<table>
<thead>
<tr>
<th>Traits</th>
<th>Means±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (days)</td>
<td>731.39±16.53</td>
</tr>
<tr>
<td>Live weight (kg)</td>
<td>539.93±51.96</td>
</tr>
<tr>
<td>Cold carcass weight (kg)</td>
<td>311.47±33.39</td>
</tr>
<tr>
<td>Longissimus muscle area (cm²)</td>
<td>75.16±8.62</td>
</tr>
<tr>
<td>Backfat thickness (cm)</td>
<td>0.70±0.28</td>
</tr>
<tr>
<td>Meat color score</td>
<td>4.85±0.46</td>
</tr>
<tr>
<td>Fat color score</td>
<td>2.98±0.16</td>
</tr>
<tr>
<td>Marbling score</td>
<td>2.25±1.36</td>
</tr>
<tr>
<td>Texture score</td>
<td>1.86±0.35</td>
</tr>
<tr>
<td>Overall maturity</td>
<td>2.00±0.00</td>
</tr>
</tbody>
</table>

In our previous study, 57 sequence variants were identified: 14 in the promoter region, 27 in introns, 8 in exons and 8 in the 3’ flanking region in leptin in Korean cattle (Yoon et al., 2005). In this study, we examined leptin gene as one of most promising candidate genes in meat production and quality in cattle. Here, we genotyped 11 exons and 8 in the 3’ flanking region in leptin in Korean cattle. The degree of marbling was evaluated according to the Korean Beef Marbling Standard, and the scores for meat color and fat color were determined using the color standard (APGS, 1995). The scores for texture and maturity were also determined using the APGS reference index.

The yield grades for carcasses were determined by three traits: the fat thickness, the area of the longissimus muscle, and the cold carcass weight. Backfat thickness was evaluated in terms of thickness of fat over the longissimus muscle measured perpendicular to the outside surface at a point two-thirds of the length of the ribeye from the chine bone end. The area of the ribeye was determined at the surface using a grid (APGS, 1995). The carcass traits are summarized in Table 1.

**Genotyping by single-base extension (SBE) and electrophoresis**

For genotyping of polymorphic sites, amplifying and extension primers were designed for single-base extension (SBE) (Vreeland et al., 2002). Primer extension reactions were performed with the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems). To clean up the primer extension reaction, one unit of SAP (shrimp alkaline phosphatase) was added to the reaction mixture, and the mixture was incubated at 37°C for 1 h, followed by 15 min at 72°C for enzyme inactivation. The DNA samples, containing extension products and Genescan 120 Liz size standard solution were added to Hi-Di formamide (Applied Biosystems) according to the recommendation of the manufacturer. The mixture was incubated at 95°C for 5 min, followed by 5 min on ice, and then electrophoresis was performed using the ABI Prism 3100 Genetic Analyzer. The results were analyzed using the program of ABI Prism GeneScan and Genotyper (Applied Biosystems). Information regarding the primers is available on our website (http://www.snp-genetics.com/user/additional_list.asp).

**Animals and phenotypic data**

The Korean cattle genomic DNA samples were obtained from 428 steers produced from 76 sires used in progeny testing program of National Livestock Research Institute (NLRI) of Korea. The dams were inseminated randomly with young sires. All steers were fed for 2 years period under tightly controlled feeding program in Daekwanryeong and Namwon branch of NLRI. All carcasses were evaluated following 24-h chill postmortem, and traits used to determine APGS (Animal Production Grading Service of Korea) quality grades (marbling, meat color, fat color, texture, overall maturity score, cold carcass weight, fat thickness, and longissimus muscle area) were recorded. Live weights were determined immediately before slaughter. After a 24-h chill, cold carcass weights were measured, and then the left side of each carcass was cut between the last rib and the first lumbar vertebrae to determine quality grade.

The quality grade was determined by assessing the degree of marbling and firmness in the cut surface of the ribeye, in relation to the maturity and fat color of the carcass. The degree of marbling was evaluated according to the Korean Beef Marbling Standard, and the scores for meat and fat color were determined using the color standard (APGS, 1995). The scores for texture and maturity were also determined using the APGS reference index.

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**MATERIALS AND METHODS**

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**Statistics**

We examined a widely used measure of linkage disequilibrium between all pairs of biallelic loci, lewontin’s d’ (hedrick, 1987), and r². haplotypes and their frequencies were inferred using the algorithm developed by Stephens et al. (2001), which (PHASE) uses a bayesian approach incorporating a priori expectations of haplotypic structure from population genetic and coalescent theory. Phase probabilities of each site were calculated for each individual using this software. Association analyses with carcass traits were performed using regression models (codominant (minor allele homozygotes vs. heterozygotes vs. major allele homozygotes), dominant (minor allele homozygotes plus heterozygotes vs. Major allele homozygotes), and recessive (minor allele homozygotes vs. heterozygotes plus major allele homozygotes)) controlling sire and age (days, continuous value) as covariates. The effective number of independent marker loci in leptin was calculated to correct for multiple testing. The effective number in leptin was calculated using the software SNPSpD.

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which is based on the spectral decomposition (SpD) of matrices of pair-wise LD between SNPs. The resulting number of independent marker loci was applied to correct for multiple testing (Nyholt, 2005). The mRNA folding structures were predicted by using MFOLD (www.bioinfo.rpi.edu/applications/mfold) (Zuker, 2003).

RESULTS AND DISCUSSION

Among previously identified 57 polymorphisms (Yoon et al., 2005), 11 polymorphisms (c.-61-575G>A, c.-61-500insdelC, c.-61-484G>A, c.-61-292C>T, c.-61-211A>G, c.-61-170C>T, c.+73C>T (R25C), c.+150C>G (S50S), c.+239C>T (A80T), c.+411T>C (A137A) and c.+495C>T (P165P)) in leptin were selected for larger-scale genotyping based on locations (promoter and coding exons). By pair-wise linkage analysis with 437 Korean cattle, which were used for genotyping, we have found that 11 sets of polymorphisms were in almost complete LDs (see Figure 1C). Three major haplotypes (freq.>0.1) identified, hl1 was not used for further analysis because it was almost (>97%) tagged by c.+73C>T (R25C) (Figure 1B). The carcass traits are summarized in Table 1.

Statistical analysis revealed that one single nucleotide polymorphism in coding exon (c.+411T>C (A137A)) showed a significant association with the quantity trait, cold carcass weight (CWT). The C-bearing genotypes (C allele homozygotes or CT heterozygotes of c.+411T>C (A137A) and c.+495C>T (P165P)) in leptin were selected for larger-scale genotyping based on locations (promoter and coding exons). By pair-wise linkage analysis with 437 Korean cattle, which were used for genotyping, we have found that 11 sets of polymorphisms were in almost complete LDs (see Figure 1C). Three major haplotypes (freq.>0.1) were constructed (Figure 1B) using these 11 polymorphisms. The minor allele frequencies of genotyped polymorphisms were 0.295 (c.-61-575G>A), 0.301 (c.-61-500insdelC), 0.228 (c.-61-484G>A), 0.392 (c.-61-292C>T), 0.294 (c.-61-211A>G), 0.232 (c.-61-170C>T), 0.205 (c.+73C>T (R25C)), 0.132 (c.+150C>G (S50S)), 0.329 (c.+239C>T (A80T)), 0.408 (c.+495C>T (P165P)) in Korean cattle (Figure 1A and Table 2). Associations of leptin polymorphisms with the carcass traits were analyzed using multiple regression models. Among three major haplotypes (freq.>0.1) identified, hl1 was not used for further analysis because it was almost (>97%) tagged by c.+73C>T (R25C) (Figure 1B). The carcass traits are summarized in Table 1.

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In analysis with marbling score (Marb), c.+150C>G (S50S) in coding exon also showed a significant association, e.g., lowest in G allele homozygotes of c.+150C>G (S50S) (c.+495C>T (P165P)) in Korean cattle (Figure 1A and Table 2).

Figure 1. Gene map, haplotypes, and LD coefficients in leptin. A. Gene map and polymorphisms in leptin on bovine chromosome 4. Coding exons are marked by black blocks and 5' and 3' UTRs by white blocks. First base of translation site is denoted as nucleotide +1. B. Haplotypes of leptin. Others contain rare haplotypes (freq.<0.01) C. Linkage disequilibrium coefficients (|D'| and r²) among leptin polymorphisms.
Table 2. Genotypes and allele frequencies of 11 polymorphisms in leptin

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>A.A. change</th>
<th>Genotype</th>
<th>Frequency</th>
<th>Heterozygosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>c. -61-575G&gt;A</td>
<td>Promoter</td>
<td>-</td>
<td>C</td>
<td>0.295</td>
<td>0.416</td>
</tr>
<tr>
<td>c. -61-500insdelC</td>
<td>Promoter</td>
<td>del</td>
<td>insdel</td>
<td>0.301</td>
<td>0.421</td>
</tr>
<tr>
<td>c. -61-484G&gt;A</td>
<td>Promoter</td>
<td>-</td>
<td>G</td>
<td>0.228</td>
<td>0.352</td>
</tr>
<tr>
<td>c. -61-292C&gt;T</td>
<td>Promoter</td>
<td>-</td>
<td>C</td>
<td>0.392</td>
<td>0.477</td>
</tr>
<tr>
<td>c. -61-21A&gt;G</td>
<td>Promoter</td>
<td>-</td>
<td>A</td>
<td>0.294</td>
<td>0.415</td>
</tr>
<tr>
<td>c. -61-170C&gt;T</td>
<td>Promoter</td>
<td>-</td>
<td>C</td>
<td>0.232</td>
<td>0.356</td>
</tr>
<tr>
<td>c. +73C&gt;T (R25C)</td>
<td>Exon2</td>
<td>Arg25Cys</td>
<td>C</td>
<td>0.205</td>
<td>0.326</td>
</tr>
<tr>
<td>c. +150C&gt;G (S50S)</td>
<td>Exon3</td>
<td>Ser50Ser</td>
<td>C</td>
<td>0.132</td>
<td>0.230</td>
</tr>
<tr>
<td>c. +293C&gt;T (A80V)</td>
<td>Exon3</td>
<td>Ala80Val</td>
<td>C</td>
<td>0.055</td>
<td>0.104</td>
</tr>
<tr>
<td>c. +411T&gt;C (A137A)</td>
<td>Exon3</td>
<td>Ala137 Ala</td>
<td>T</td>
<td>0.329</td>
<td>0.441</td>
</tr>
<tr>
<td>c. +495C&gt;T (P165P)</td>
<td>Exon3</td>
<td>Pro165Pro</td>
<td>C</td>
<td>0.408</td>
<td>0.483</td>
</tr>
</tbody>
</table>

Table 3. Regression analyses of leptin polymorphisms with meat trait (cold carcass weight and marbling score) among Korean cattle

<table>
<thead>
<tr>
<th>Trait</th>
<th>Genotype</th>
<th>Analyzing model</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWT</td>
<td>Genotype</td>
<td>Codom</td>
</tr>
<tr>
<td>Cold carcass weight</td>
<td>C/C*</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>C/R*</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>R/R*</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Rec</td>
<td>0.18</td>
</tr>
<tr>
<td>Marb</td>
<td>Genotype</td>
<td>Codom</td>
</tr>
<tr>
<td>Marbling score</td>
<td>C/C*</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>C/R*</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>R/R*</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>Rec</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Genotypes and haplotype distributions, means, standard deviations (SD) and P-values of three alternative models are shown.
* C/C, C/R and R/R represents for common allele, heterozygotes and homozygotes for rare allele, respectively.

(Marb = 1.58), intermediate in CG heterozygotes (Marb = 1.29), and highest in C allele homozygotes (Marb = 2.27) (p < 0.01). In further haplotype association analysis, no association was detected with CWT or Marb (Table 3).
The regulation of mRNA decay is an important control point of gene expression, and is mediated by a subset of sequence elements, factors, and endoribonucleases (Wilusz et al., 2001). The mechanisms by which these coding region mRNA stability/instability elements affect mRNA decay are complex. As one of tools to examine possible differences mediated by alternative alleles of two polymorphisms (c.+150C>G [S50S] and c.+411T>C [A137A]), mRNA stability/instability were predicted using software, MFOLD (Zuker, 2003), which predicts a minimum free energy, as well as minimum free energies for foldings that must contain any particular base pair. Although the results were only predicted by using software, obvious changes in folding patterns of mRNA were detected among alternative alleles of c.+150C>G and c.+411T>C of leptin mRNAs (Figure 2).

Adipocytes are the principal site of leptin production in cattle (Ji et al., 1998). Adipocytes store excess energy in the form of triglycerides when energy intake exceeds that which is needed for homeostasis and will subsequently release free fatty acids when dietary energy is inadequate (Kim and Moustaid-Moussa, 2000). Total adipose tissue mass increases via replication and differentiation of preadipocytes. Adipose tissue mass is influenced by volume

Figure 2. Leptin mRNA folding structures, predicted by MFOLD. Output plots predicted by MFOLD (http://www.bioinfo.rpi.edu/applications/mfold). Alternative alleles were used for each model. A. Wild type leptin mRNA folding structure (c.+150C and c.+411T). Alternative folding models by single nucleotide change(s), B. c.+150G, C. c.+411C, D. c.+150G and c.+411C. The sequence, NM_173928 was used for predicting mRNA folding structure of leptin.
and number of adipocytes (Prins and O’Rahilly, 1997). As adipocytes increase in mass, peripheral concentrations of leptin increase (Considine, 1997; Ahima and Flier, 2000). Leptin concentration is significantly associated with carcass composition (marbling, backfat depth, and heart fat) and quality grade in cattle, and provide an additional indicator of fat content in feedlot cattle (Geary et al., 2003). These results are consistent with the association of polymorphisms (c.+150C>G (S50S) and c.+411T>C (A137A)) in leptin with carcass traits (CWT and Marb).

In previous study, leptin mRNA expression was higher in T allele homozygotes of c.+73C>T (R25C) and this common polymorphism was associated with fat content in adipose tissue and milk yield (Buchanan et al., 2002; Buchanan et al., 2003). But in our study with beef cattle, c.+73C>T (R25C) showed no association with CWT and Marb, although examination with milk yields were not available.

The effects of leptin polymorphisms on the carcass traits were not dramatic in the present study. When corrections for multiple testing were strictly adopted, associated P-values could not retain the significances (the threshold of 0.0054). However, further biological and/or functional evidence would be needed to confirm the suggestive associations of leptin polymorphisms with carcass traits in this study. The information for genetic involvement of c.+411T>C (A137A) and c.+150C>G (S50S) in the carcass quantity and quality (CWT and Marb) may be an important genetic information to breeders and meat producers.

ACKNOWLEDGEMENTS

This work was supported by a grant from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

REFERENCES


