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Effects of genetic variants in the promoter region of the bovine adiponectin (*ADIPOQ*) gene on marbling of Hanwoo beef cattle

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ABSTRACT

This study aimed to verify genetic effects of the bovine adiponectin (*ADIPOQ*) gene on carcass traits of Hanwoo cattle. The measured carcass traits were marbling score (MAR), backfat thickness (BFT), loineye area (LEA), and carcass weight (CAW). Selection of primers was based on the bovine *ADIPOQ* sequence, and the analysis amplified approximately 267 and 333 bp genomic segments, including 67 bp of insertions in the promoter region. Sequencing analysis confirmed genetic variants (g.81966235C > T, g.81966377 T > C, and g.81966364D > I) that showed significant effects on MAR. The present results suggest that the identified SNPs are useful genetic markers for the improvement of carcass traits in Hanwoo cattle.

1. Introduction

Many studies have tried to find significant associations between quantitative trait loci (QTL) and genetic markers identified from the whole genome and focused on finding causative genetic variants related to production traits of beef cattle (Casas et al., 2000; Davis et al., 1998; Gao et al., 2007; Li et al., 2004). Carcass, fat, and meat quality traits are critical factors for determination of quality grades of meat products in the commercial beef industry. If genetic variants explain phenotypic variations of QTLs, the utilization of genetic markers in marker assisted selection (MAS) programs should be a potential candidate approach. However, a limited number of genetic markers, which often explain a relatively small proportion of the genetic variation for QTLs (Dekkers, 2004), have been developed for carcass and meat quality traits in beef.

As a member of the adipocytokine family, *ADIPOQ*, which is the most abundant protein secreted by white adipose tissue in mammalian species, influences lipogenesis, glucose genesis, insulin sensitivity, inflammatory processes, and cardiovascular functions (Pineiro et al., 2005; Scherer, Williams, Fogliano, Baldini, & Lodish, 1995; Yokota et al., 2002). *ADIPOQ*, a signaling molecule with 247 amino acids, has either a full-length or a globular form of proteins according to the proteolytic cleavages with disulphide bonds (Yokota et al., 2002). The full-length form tends to decrease glucose, whereas the globular form stimulates oxidation in muscle. Recent studies argued that *ADIPOQ*, which is also produced in brown adipose tissue (Kadowaki & Yamauchi, 2005; Morsci, Sellner, Schnabel, & Taylor, 2006), enhances fatty acid oxidation and associates with fatty acid binding proteins (Dall'Olio, Roberta, Buttazzoni, Zambonelli, & Vincenzo, 2009; Wei et al., 2013). The functional roles described above are related to fat mechanisms, and a human study suggests that *ADIOPQ* is associated with obesity and diabetes (Hsueh et al., 2003). Another study also revealed significant functional roles of *ADIPOQ*, demonstrating that the expression levels are associated with obesity in humans (Kubota et al., 2002). In addition, human medical science tried to show phenotypic associations of obesity, diabetic susceptibility, and metabolic phenotypes with the genetic variations of *ADIPOQ* (Melistas et al., 2009; Szopa et al., 2009).

In contrast to human studies, analyses of animal data focused on finding associations of carcass traits with genetic variants in genomic regions of *ADIPOQ*. As a result, QTL analyses regarding loin eye area (LEA) and back-fat thickness (BFT) in cattle have shown several genetic variants of the *ADIPOQ* gene in Angus, Korean, and Chinese cattle (Morsci et al., 2006; Shin & Chung, 2013; Zhang et al., 2009). Recent evidence suggests that secretion of *ADIPOQ* is negatively correlated with adipose tissue mass (Kadowaki & Yamauchi, 2005) and is detected in skeletal muscle (Morsci et al., 2006), indicating that *ADIPOQ* is correlated with the regulation of lipid and carbohydrate metabolism. In addition, a study found that *ADIPOQ* influences yield grade and weight traits in cattle (Morsci et al., 2006).

Location of the bovine *ADIPOQ* gene was revealed to be on bovine chromosome 1 (BTA1) near QTLs for marbling scores (MAR), meat quality grade, and LEA (Cai et al., 2004). Polymorphisms in the *ADIPOQ* gene in pigs were also reported to be associated with fatty acid binding protein (*FABP4*), fat deposition, carcass traits (Dai et al., 2006; Dall'Olio et al., 2009; Wei et al., 2013), and reproductive traits (Houde, Murphy, Mathieu, Bordignon, & Palin, 2008).

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The abundant marbling found in Hanwoo cattle has become an important factor influencing the quality of meat products in commercial beef markets. In general, studies focused on associations between genetic variants and fat traits using well-constructed Hanwoo populations that made it easy to trace allele effects. However, it is necessary to verify marker effects in random beef populations. If marker effects are confirmed, the alleles may be used as standardized molecular markers to maximize production rates of herds. Therefore, the present analysis tried to find significant associations based on genetic variants of *ADIPOQ* for various carcass traits using a random beef population from the entire nation without considering paternal or maternal genetic effects.

2. Materials and methods

2.1. Animals and Carcass traits

The ethics and welfare committee of the National Institute of Animal Science (NIAS) approved this experiment. A total of 1,954 Hanwoo cattle were randomly collected from the entire nation with help of the Nonghyup Hanwoo Cooperation in Korea during 2012 and 2013. Meat samples from Hanwoo cattle that were registered in the national database were randomly collected from 9 packing facilities of Korean Animal Products Evaluation (KAPE), which is an official Korean grader for meat quality grades. The meat quality of Hanwoo cattle was based on a grading system (http://www.ekape.or.kr/view/eng/system/beef.asp) from KAPE. The data collected by KAPE were carcass weight (CAW, kg), loineye area (LEA, cm^2), backfat thickness (BFT, cm), and marbling score (MAR, ranged from 1 [poor] to 9). Approximately 5 g of muscle samples were collected from the longissimus *thoracis* muscle around the 6th rib 24 hours after slaughter, and stored at −70 °C.

2.2. Genomic DNA preparation

For the extraction of genomic DNA, approximately 1 gram of muscle sample was used. After chopping the samples briefly, the pieces were placed into a tube with an extraction buffer, and genomic DNAs were extracted using a commercial kit (Wizard DNA extraction kit, Promega) according to the manufacturer's guidelines. DNA quantity and purity (A260/A280 ratio) for each sample were assessed using the NanoDrop 1000 spectrophotometer (Thermo Scientific, USA), and the genomic DNAs were stored in a −70 °C freezer until genotyping.

2.3. Amplification

Primers were based on the bovine sequence (GenBank accession number JQ775868) and designed using the DNAselect program of DNAstar version 6.0. To select appropriate primer sets that can be used for all cattle breeds, alignments were performed with 6 reference sequences from GenBank (accession numbers DQ156119, EU296533, EU313339, EU492456, EU492457, and JQ775868). Eight potential SNPs (g.81965420G > C, g.81965993G > C, g.81966286C > A, g.81966 690A > G, g.81966775 T > C, g.81966798G > A, g.81966940A > G, and g. 81967160 T > C) were found. The present analysis selected a SNP position (g.81966377 T > C) that may have been identified in many cattle breeds according to the literature (Morsci et al., 2006; Shin & Chung, 2013; Zhang et al., 2013). Therefore, the primer set focused on the genomic region (81966163–81966429, approximately 267 bp) that contained the SNP, and the forward and reverse primers were GCAGC TCTAC TTGGC ATCC (nucleotide positions 81966163–81966184) and CTTGA ATCAG TCGTC CTTAC CC (81966410–81966429), respectively. The alignment found a 67 bp insertion inside the priming region, and, therefore, the final amplification products were expected to be 267 bp and 333 bp using the same primer set.

Two microliters of 10 X reaction buffer (10 mM Tris, pH 8.3, 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$), 2.5 mM dNTP, 10 pmol of each primer, 50 ng of genomic DNA, and 1 unit of *Taq* DNA polymerase (Gibco BRL, Grand Island, NY) in a final volume of 20 ul were used. After heating at 95 °C for 2 min, a total of 35 cycles were adapted for denaturation at 94 °C/1 min, annealing at 59 °C/1 min, and polymerization at 72 °C/1.5 min (MJ Research, PT-200, Watertown, MA). DNA bands in the agarose gels were stained with ethidium bromide and were visualized with UV light.

2.4. Detection of single nucleotide polymorphism

After PCR, DNA samples were purified using the PCR purification system (Nucleogen, Korea) to perform direct sequencing analyses for all samples with an ABI3730 XL Genetic Analyzer (Applied Biosystems) at NIAS. The direct sequencing analysis was performed for all samples with both forward and reverse primers to minimize base calling errors. The conditions for sequencing analysis were 94 °C/10 sec and 60 °C/ 4 min for 35 cycles in a total volume of 10 ul (5 ul of sequencing buffer, 1.6 pM primer, and 50 ng of the amplified DNA, 0.5 ul Bigdye terminator, and distilled water). A purification procedure for sequencing products was conducted with 75% Isopropanol and 70% Ethanol for centrifugation at 2,800 rpm for 45 min. After drying the samples at room temperature for 2 hours, deionized formamide (10 ul) was added. The alignment for all individual sequences was conducted with the SeqMan program of DNAstar version 6.0, and genotypes were determined according to the peaks of sequence diagrams. For the genotyping to determine insertion or deletion, 1.5% agarose gel electrophoresis was conducted, and genotypes were determined based on banding patterns of DNA for fast (267 bp, Deletion) and slow (333 bp, Insertion) mobilities.

2.5. Statistical analysis

Analysis of variance was conducted using the Statistical Analysis System (SAS, 2012) with general linear model (GLM) procedures to investigate effects of the genotypes on carcass traits. Least squares means were compared using Fisher's least significant difference test with a comparison error rate of 0.05. Additive genetic effects were estimated by the difference between estimates for the two homozygous genotypes, and the dominance deviation was estimated by the difference between the solution for the heterozygous genotype and the average of the solutions for the two homozygous genotypes. Least squares means and standard errors for each trait were estimated using a linear model as follows: $Y = \mu + G + e$, where Y is the observation of traits, μ is the overall mean for each trait, G is the fixed effect of genotype, and e is the residual error. Allele frequencies and Hardy-Weinberg equilibrium were estimated using Arlenquin version 3.0. Linkage disequilibrium in a 333 bp genomic region was estimated using the Haploview software. The haplotypes were constructed with g.81966235C > T, g.81966364D > I, and g.81966377 T > C.

3. Results

3.1. Descriptive summary of carcass traits

Table 1 presents a descriptive summary of the carcass traits (MAR, BFT, LEA, and CAW), reporting measurement units, means with

Table 1
Descriptive summary of carcass traits for Hanwoo cattle.

Item	Marbling (MAR, 1–9)	Backfat thickness (BFT, mm)	Loineye area (LEA, cm^2)	Carcass weight (CAW, kg)
Mean	5.9	11.9	90.1	427.1
SD	1.7	4.0	9.5	47.0
Minimum	1	4	61	286
Maximum	9	29	125	579
Normality	0.002	0.051	0.285	0.206

[1]MAR: marbling scores (1, low to 9, high), BFT: backfat thickness, LEA: loineye area, and CAW: carcass weight.
Normality was based on the chi-square distribution.