

## EFFECTS OF CALPASTATIN GENE POLYMORPHISMS ON GROWTH AND CARCASS TRAITS OF KOREAN NATIVE CATTLE

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### INTRODUCTION

Understanding the biochemical and physiological mechanisms underlying growth is one of the major subjects of interest in the cattle industry. Several studies have investigated the growth factors and growth hormones that influence growth. However, it is still unclear which genes and how many genes are involved in the process of growth. Calpastatin, which is an endogenous protease inhibitor (EC 3.4.22.17, Ca<sup>2+</sup> dependent cysteine proteinase), has been studied (Goll *et al.*, 1992), along with several growth factors, to investigate their effects on growth, and on cell growth and differentiation (Temm-Grove *et al.*, 1999). Cottin *et al.* (1994) reported that the calpain system may influence cell growth. If the calpastatin system is highly heritable, and has moderate to high genetic correlations with economic traits of interest, its use in a selection program may increase the rate of genetic change. Carcass traits are often used to evaluate meat quality. Marbling is one of the major traits used in evaluation of meat quality (Wulf *et al.*, 1996). Significant relationships among carcass traits, and between the calpain system and meat tenderness (Shackelford *et al.*, 1994), have been reported. Carcass traits are influenced by genetic variations, and genetic differences in tenderness, marbling and calpastatin activity have been reported among and within breeds of cattle. Therefore, this study was carried out to investigate the effects of calpastatin genotypes on weight and carcass traits to provide genetic information for improvement of these traits.

### MATERIALS AND METHODS

**Animals.** Hundred-thirty-one Korean native cattle, which were part of the 28th progeny test in 1999, were used from Daekwanryung and Namwon branches of the National Livestock Research Institute (NLRI). The cattle were fed a postweaning corn and soybean meal diet, which was formulated to meet NRC (1984) requirements for growing beef cattle.

**Traits measured.** Weights were recorded at 3 (WT3), 6 (WT6), 12 (WT12), and 24 (WT24) mo of age. The following carcass measurements were obtained: backfat thickness (BAF) between the 12<sup>th</sup> and 13<sup>th</sup> rib; dressing percentage (DRP); loineye area (LEA), and marbling score (MAR) (1= grade 3, 2-3= grade 2, 4-5= grade 1, and 6-7= 1<sup>+</sup>).

**Design of primers.** PCR primers for CAST67 (exons 6 and 7) and CAST28 (domain IV) were designed based on the bovine calpastatin cDNA (Killefer and Koohmaraie, 1994; GenBank accession no. L14450). Forward and reverse primer sequences of CAST67 were AGCAG CCACC ATCAG AGAAA, and TCAGC TGGTT CCGCA GAT, respectively. Forward and reverse primer sequences of CAST28 were GTGCC CAGGA CCCCA TTG, and AGCAG

GCTTC TTGTC TTTGT C, respectively.

**Polymerase chain reaction.** PCR was conducted with a final volume of 30  $\mu$ l, including 3  $\mu$ l of 10 X reaction buffer (10 mM Tris, pH 8.3, 50 mM KCl, 0.1% Triton X-100, 1.5 mM  $MgCl_2$ ), 10  $\mu$ M dNTP, 10 pM of each primer, 50 ng of genomic DNA, and two units of Taq DNA polymerase. After denaturation for 2 min at 95°C, PCR cycles for CAST67 were adapted to 94°C for 1 min for denaturation, 57°C for 1 min for annealing, and 72°C for 1.5 min for polymerization (Perkin Elmer, 2400). Conditions for CAST28 were 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, with a total of 35 cycles (Eric Comp, 200).

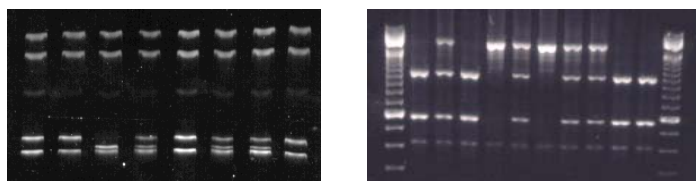
**Sequencing.** Sequencing of the PCR products was conducted with an ABI3100 machine at the Genetic Resources Center of the National Livestock Research Institute (Chungnam, Korea).

**Statistical analysis.** Least squares means and standard errors were determined for all measurements with a model including fixed effects of calpastatin genotypes, age of dam, and location (Daekwanryung and Namwon), and a covariate for age of animal. Analysis of variance was conducted using Statistical Analysis System (SAS) general linear models (GLM) procedures, and least squares means were compared using Fisher's least significant difference test (SAS, 1985) with a comparison error rate of 0.05.

## RESULTS AND DISCUSSION

**Genetic variants.** Genetic variants for calpastatin domains IV (CAST28) and L (CAST67) have been reported for the bovine (Chung, 2001). Polymorphisms in the bovine calpastatin segments for the non-coding regions of domains IV and L were detected in this study. Shown in Figure 1 are genetic variants for CAST28 and CAST67 segments, which were analyzed using single strand conformation polymorphism (SSCP) with Taq I restriction enzyme and restriction fragment length polymorphism (RFLP) with Xmn I restriction enzyme, respectively. Allele frequencies were calculated as 0.27 and 0.73 for the A and B allele of CAST28, and 0.67 and 0.33 for the A and B allele of CAST67, respectively. These results were similar to those found in a previous report involving an Angus population (Chung, 2001).

**Effects of genotypes.** Genetic variants of CAST28 and CAST67 segments found in this study did not account for significant variation in weight (Table 1). These results were similar to those of Chung *et al.* (1999) who found no significant differences among CAST genotypes for weight traits in an Angus population. However, they reported that genotypes for the calpain I large subunit explained some variation in weight traits. According to Cottin *et al.* (1994), the calpain system was detected in the early cell culture growth stage. The calpain system may be a cofactor for increasing muscle differentiation. Hitomi *et al.* (2000) also suggested that the calpain system, including calpastatin, is associated with the initial stages of cell differentiation. Therefore, the calpain system may be involved in the process of growth at early embryonic developmental stages or late skeletal muscle developmental stages.



**Figure 1.** PCR-SSCP polymorphism for CAST28 (left) segment in bovine calpastatin domain IV after digestion with Taq I at 65°C for 2 h. SSCP was conducted at 10°C for 14 h using 0.5 X MDE gels, and RFLP for CAST67 (right) in bovine calpastatin domain L was conducted with Xmn I at 37°C for 2 h. Three genotypes (*AA*, *AB*, and *BB*) are demonstrated

**Table 1.** Least squares means and standard errors for weight traits by genotypes

Segment Genotype	WT3, kg	WT6, kg	WT12, kg	WT24, kg
CAST28	P=0.86	P=0.77	P=0.92	P=0.90
AA	163.6 ± 6.7	269.0 ± 8.8	430.9 ± 9.3	538.1 ± 11.4
AB	163.9 ± 5.9	279.0 ± 7.7	430.9 ± 8.1	535.6 ± 10.0
BB	162.7 ± 5.6	275.1 ± 7.3	433.0 ± 7.7	540.7 ± 7.4
CAST67	P=0.61	P=0.89	P=0.93	P=0.99
AA	157.6 ± 6.5	270.1 ± 8.8	430.4 ± 9.3	537.4 ± 10.9
AB	167.9 ± 7.4	278.0 ± 9.9	429.7 ± 8.1	540.2 ± 12.5
BB	165.7 ± 6.6	274.4 ± 8.9	434.2 ± 7.7	539.0 ± 11.1

P : probability

**Table 2.** Least squares means and standard errors for carcass traits by genotypes

Segment Genotype	DRP, %	LEA, cm <sup>2</sup>	BAF, cm	MAR(1-7)
CAST28	P=0.84	P=0.28	P=0.02	P=0.63
AA	57.55 ± 0.4	78.0 ± 1.9	0.72 ± 0.04 <sup>a</sup>	2.9 ± 0.4
AB	57.33 ± 0.3	82.6 ± 1.7	0.73 ± 0.05 <sup>a</sup>	2.6 ± 0.3
BB	57.56 ± 0.3	81.4 ± 1.6	0.94 ± 0.03 <sup>b</sup>	3.1 ± 0.3
CAST67	P=0.47	P=0.95	P=0.68	P=0.26
AA	57.64 ± 0.3	79.8 ± 1.9	0.84 ± 0.08	3.3 ± 0.4
AB	57.54 ± 0.4	81.0 ± 2.1	0.77 ± 0.09	2.7 ± 0.4
BB	57.12 ± 0.3	80.0 ± 1.9	0.78 ± 0.08	2.5 ± 0.4

Calpastatin genotypes did not account for significant variation in any of the carcass traits except BAF (Table 2). The least squares mean of the BB genotype (0.94 cm) in CAST28 segment for BAF was significantly different from those of the AA (0.72 cm) and AB (0.73 cm) genotypes. Morgan *et al.* (1993) stated that decreased 12th rib fat thickness could result in greater chilling rate, and, thus, a decreased rate of decline in calpastatin activity in meat from bulls. Based on the significant relationship between calpastatin activity and calpastatin

genotypes (Chung *et al.*, 1999), calpastatin activity can be classified by calpastatin genotypes. Thus, classified calpastatin genotypes can be used to determine level of backfat thickness. Our strong association between BAF and calpastatin genotypes ( $P < 0.02$ ) was consistent with this hypothesis.

#### CONCLUSION

Genotypes for CAST28 significantly influenced BAF, but genotypes for CAST67 did not explain significant variation in weight or carcass traits. BB genotypes for CAST28 had the highest BAF values (BB>AB>AA). Significant associations with location were found for all of the weight and carcass traits, which was expected. Our findings indicate that calpastatin segments may be used in marker assisted selection programs to reduce backfat thickness (BAF). Consequently, results of the present study, and future genotypic data from these animals, based on variation in the calpastatin loci, will provide critical information for establishing the calpain family as a source of candidate genes.

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