Effects of *calpastatin* and μ-*calpain* markers in beef cattle on tenderness traits[1,2]

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ABSTRACT: The objective of this study was to assess the association of single nucleotide polymorphisms (SNP) developed at the *calpastatin* (*CAST*) and μ-*calpain* (*CAPN1*) genes with meat tenderness and palatability traits in populations with diverse genetic backgrounds. Three populations were used in the study. One population consisted of *Bos taurus* that included crossbred animals derived from Hereford, Angus, Red Angus, Limousin, Charolais, Gelbvieh, and Simmental (GPE7; n = 539). Another population consisted of *Bos taurus* with *Bos indicus* influence, including crossbred animals from Hereford, Angus, Brangus, Beefmaster, Bonsmara, and Romosinuano (GPE8; n = 580). The third population was *Bos indicus* and consisted of purebred Brahman (STARS; n = 444). Traits evaluated were meat tenderness measured as Warner-Bratzler shear force (WBSF; kg) at 14 d postmortem, and traits evaluated by trained sensory panels that included tenderness score, juiciness, and flavor intensity. A SNP at the *CAST* gene had a significant ($P < 0.003$) effect on WBSF and tenderness score in the GPE7 and GPE8 populations. Animals inheriting the TT genotype at *CAST* had meat that was more tender than those inheriting the CC genotype. The marker at the *CAPN1* gene was significant ($P < 0.03$) for tenderness score in GPE7 and GPE8. Animals inheriting the CC genotype at *CAPN1* had meat that was more tender than those inheriting the TT genotype. Markers at the *CAST* and *CAPN1* genes were associated with flavor intensity in the GPE8 population. Animals inheriting the CC genotype at *CAST* and the TT genotype at *CAPN1* produced steaks with an intense flavor when compared with the other genotypes. An interaction between *CAST* and *CAPN1* was detected ($P < 0.05$) for WBSF on GPE8. The statistical significance of the interaction is questionable because of the limited number of observations in some cells. Markers developed at the *CAST* and *CAPN1* genes are suitable for use in identifying animals with the genetic potential to produce meat that is more tender.

Key words: μ-*calpain*, *calpastatin*, cattle, genetic marker, meat tenderness, shear force

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INTRODUCTION

Meat tenderness is one of the most important factors leading to consumer satisfaction when eating beef. Among the factors that have been identified as responsible for the postmortem meat tenderization process is the calpain proteolytic system. Two enzymes responsible for this process are the micromolar calcium-activated neutral protease μ-*calpain* (***CAPN1***), which is encoded by the *CAPN1* gene, and its inhibitor, *calpastatin* (***CAST***), which is encoded by the *CAST* gene (Koohmaraie, 1996).

To date several markers have been developed at the *CAST* gene (Barendse, 2002), and 3 markers have been developed at the *CAPN1* gene (Page et al., 2002; White et al., 2005). Previous studies (Barendse, 2002; Page et al., 2002, 2004; White et al., 2005) have independently evaluated markers at the *CAST* and *CAPN1* genes. These studies have shown an association of individual markers at *CAST* and *CAPN1* with meat tenderness in beef cattle. However, there has been no simultaneous evaluation of both genes to assess their effect in meat tenderization. Thus, the objective of the study was to

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assess the association of markers at the *CAST* and *CAPN1* genes with meat tenderness in populations with diverse genetic backgrounds.

MATERIALS AND METHODS

Populations

Three independent populations were studied. The first was a population of *Bos taurus* descent, the second was a population that included germplasm from *Bos taurus* and *Bos indicus,* and the third was a purebred *Bos indicus* population.

Cycle 7 of the Germplasm Evaluation project (**GPE7**) included 539 crossbred steers of *Bos taurus* descent that were used in this study (Page et al., 2004; Wheeler et al., 2005). In brief, approximately equal numbers of calves were produced from 149 purebred sires representing the 7 beef breeds in the United States with the highest numbers of annual registrations (Hereford, Angus, Red Angus, Simmental, Gelbvieh, Limousin, and Charolais). These sires were mated to Angus, Hereford, or MARCIII (composite of ¼ Hereford, ¼ Angus, ¼ Pinzgauer, and ¼ Red Poll) cows. Management of cattle and collection of phenotypic data have been recently described by Wheeler et al. (2005).

Cycle 8 of the Germplasm Evaluation project (**GPE8**) included 580 crossbred steers that were used in this study (T. L. Wheeler, personal communication). Briefly, approximately equal numbers of calves were produced from 127 purebred sires representing tropically adapted breeds, including Beefmaster, Brangus, Bonsmara, and Romosinuano, as well as Hereford and Angus. All dams were Angus or MARCIII cows. Management of these animals and collection of phenotypes were similar to GPE7 (T. L. Wheeler, personal communication).

A population of 504 Brahman calves managed by the Subtropical Agricultural Research Station (Brooksville, FL) and collection of phenotypic data have been previously described (Riley et al., 2002) and will be referred herein as the **STARS** population. Briefly, 22 sires were used over 5 yr to produce Brahman calves in 1996 through 2000 (246 steers; 258 heifers). Calves were fed on site and were slaughtered at a commercial facility in Florida.

Traits Evaluated

Traits analyzed were meat tenderness measured as Warner-Bratzler shear force (**WBSF**), tenderness score, juiciness, and flavor intensity. Warner-Bratzler shear force data were collected on LM samples from steers at d 14 postmortem for GPE7 and GPE8 (Wheeler et al., 2005) and from steers and heifers at d 14 postmortem for STARS (Riley et al., 2003). Wheeler et al. (2005) and Riley et al. (2003) describe the method for obtaining tenderness scores, juiciness, and flavor from the steaks. In brief, 2.54 cm thick frozen steaks were thawed between 4° and 5°C during 18 to 24 h. Steaks were cooked, and samples were given to trained sensory panel members (AMSA, 1995). The panel members evaluated the steaks for tenderness, juiciness, and beef flavor on scales of 1 through 8 (1 = extremely tough, extremely dry, extremely bland; 8 = extremely tender, extremely juicy, extremely intense).

Markers Used

The single nucleotide polymorphism (**SNP**) developed at the *CAST* gene was reported by Barendse (2002). The marker is a transition from a guanine to an adenine at the 3′ untranslated region of the gene. The marker will be referred to as *CAST*.

The marker developed at the *CAPN1* gene was reported by White et al. (2005). The marker is a transition from a cytosine to a thymine at position 6545 of the GenBank accession AF248054 from the gene. The marker will be referred to as *CAPN1* (White et al., 2005).

Genotyping

For the GPE7 and GPE8 populations, a saturated salt procedure (Miller et al., 1988) was used to obtain DNA from white blood cells. For the STARS population, DNA was obtained using a Qiagen QIAmp DNA blood mini kit (Valencia, CA). Blood samples were collected in 60-mL syringes with 4% EDTA. Blood was spun at 2,500 rpm for 25 min, and buffy coats were aspirated, cleaned, and frozen until DNA was extracted (Casas et al., 2005; White et al., 2005).

Genotyping was performed using a primer extension method with mass spectrometry-based analysis of the extension products on a MassArray system as suggested by the manufacturer (Sequenom, Inc., San Diego, CA) and as described by Stone et al. (2002). A universal mass tag sequence was added to the 5′ end of each gene-specific amplification primer sequence as recommended by the manufacturer. Genotypes for each animal were collected, and the automated calls were checked by visualization of the spectrographs to minimize errors. Limited availability of tissue samples and problems with degradation of existing DNA samples hampered the collection of a complete dataset of all animals for the markers. When necessary, genotype assays were performed a second time to increase the number of successful genotypes, but samples were not tried a third time.

Statistical Methods

Model was evaluated using the Mixed procedure of SAS (SAS Inst., Inc., Cary, NC). The model used for GPE7 and GPE8 included sire breed, dam breed, the interaction between sire breed and dam breed, year of birth, slaughter group within year, *CAST* genotype, *CAPN1* genotype, and the interaction between *CAST* and *CAPN1* genotypes as fixed effects (White et al., 2005). The interaction between the *CAST* and the *CAPN1* genotypes was removed from the model when not significant. Weaning age was included as a linear

Table 1. Number of records, means, and SD for Warner-Bratzler shear force (WBSF), tenderness score, juiciness, and flavor intensity of the steak in the *Bos taurus* (GPE7), *Bos taurus* with *Bos indicus* influence (GPE8), and *Bos indicus* (STARS) populations

	GPE7			GPE8			STARS		
Trait	No.	Mean	SD	No.	Mean	SD	No.	Mean	SD
WBSF (kg)	563	4.25	0.82	608	3.79	0.73	473	5.29	1.72
Tenderness[1]	563	5.56	0.77	608	5.77	0.52	474	4.93	0.71
Juiciness[2]	563	5.30	0.31	608	5.53	0.29	474	5.25	0.55
Flavor[3]	563	4.89	0.36	608	4.62	0.39	474	5.88	0.41

[1]1 = Extremely tough; 4 = slightly tough; 5 = slightly tender; 8 = extremely tender.
[2]1 = Extremely dry; 4 = slightly dry; 5 = slightly juicy; 8 = extremely juicy.
[3]1 = Extremely bland; 4 = slightly bland; 5 = slightly intense; 8 = extremely intense.

covariate. Sire was included as a random effect nested within sire breed. The model for the STARS population included the random effect of sire, the fixed effects of contemporary group (1 through 44), *CAST* genotype, *CAPN1* genotype, and the interaction between *CAST* and *CAPN1* genotypes (Casas et al., 2005). The interaction between the *CAST* and the *CAPN1* genotypes was removed from the model when not significant. Contemporary group was defined as a group of calves of the same gender, fed in the same pen, and slaughtered on the same date. There were 44 contemporary groups in the study. Probability values were not corrected for multiple testing.

RESULTS

Phenotypic Variation

Traits evaluated, number of records, and simple statistics are presented in Table 1. All traits displayed substantial variation within population. A total of 539, 580, and 444 individuals were used for GPE7, GPE8, and STARS, respectively, in the study (Table 2). Samples from 24, 28, and 60 individuals in GPE7, GPE8, and STARS, respectively, were unable to amplify any product to be genotyped for the *CAST* assay, the *CAPN1* assay, or both. The statistics presented in Table 1 are for the total number of individuals with phenotypic information, regardless of whether the samples produced successful genotypes for both markers.

Genotype Frequencies

Table 2 shows the number of animals with the genotypes for the SNP used in the *CAST* and *CAPN1* markers. Genotypes for the CC class at *CAST* were in low frequency across populations. The frequency of the CC genotype was 4.4, 2.2, and 6.1% in the GPE7, GPE8, and STARS populations, respectively. The frequency of the C allele was 19.8, 16.7, and 28.1% for GPE7, GPE8, and STARS populations, respectively. White et al. (2005) evaluated the genotypic and allelic frequencies of *CAPN1* in these populations. The CC genotype at *CAPN1* was absent in the STARS population. Thus, comparisons were only made between CT and TT genotypes in this population. The low percentage of animals inheriting the CC genotype at the *CAST* marker generated a low frequency of allelic combinations with *CAPN1*. For example, only 3 animals inherited the CC *CAST* and the TT *CAPN1* genotype in the GPE8 population.

CAST Effect

Levels of significance, least squares means, and standard errors are reported in Table 3 for the effect of *CAST* on WBSF, tenderness score, juiciness, and flavor in the populations studied. The marker at the *CAST* gene was associated ($P < 0.01$) with WBSF and tenderness score in the GPE7 and GPE8 populations. Animals inheriting the CC and the CT genotypes produced tougher meat when compared with animals that inherited the TT geno-

Table 2. Number of individuals inheriting the CC, CT, and TT genotypes at the *calpastatin* (CAST) and μ-*calpain* (*CAPN1*) genes in the *Bos taurus* (GPE7), *Bos taurus* with *Bos indicus* influence (GPE8), and *Bos indicus* (STARS) populations

	CAPN1											
	GPE7				GPE8				STARS			
CAST	CC	CT	TT	Total	CC	CT	TT	Total	CC	CT	TT	Total
CC	9	11	4	24	4	6	3	13	—	5	22	27
CT	65	78	23	166	73	79	16	168	—	39	157	196
TT	109	167	73	349	154	195	50	399	—	41	180	221
Total	183	256	100	539	231	280	69	580	—	85	359	444

Table 3. Genotype contrasts for Warner-Bratzler shear force (WBSF) at d 14 postmortem, tenderness score, juiciness, and flavor intensity of the steak with the marker at the *calpastatin* (*CAST*) gene in the *Bos taurus* (GPE7), *Bos taurus* with *Bos indicus* influence (GPE8), and *Bos indicus* (STARS) populations

		CAST[1]		
Trait	Population	CC-TT	CT-TT	*P*-value
WBSF (kg)	GPE7	0.31 ± 0.17	0.29 ± 0.08	0.0005
	GPE8	0.48 ± 0.19	0.28 ± 0.06	<0.0001
	STARS	0.47 ± 0.26	0.05 ± 0.13	0.194
Tenderness[2]	GPE7	–0.28 ± 0.17	–0.25 ± 0.08	0.003
	GPE8	–0.31 ± 0.14	–0.16 ± 0.05	0.001
	STARS	–0.21 ± 0.14	0.03 ± 0.07	0.252
Juiciness[3]	GPE7	0.06 ± 0.06	–0.09 ± 0.03	0.002
	GPE8	–0.06 ± 0.06	–0.01 ± 0.02	0.598
	STARS	–0.06 ± 0.12	0.03 ± 0.06	0.719
Flavor[4]	GPE7	–0.001 ± 0.07	–0.03 ± 0.03	0.598
	GPE8	–0.23 ± 0.07	–0.001 ± 0.02	0.006
	STARS	–0.13 ± 0.08	–0.05 ± 0.04	0.151

[1]Mean ± SEM.
[2]1 = Extremely tough; 4 = slightly tough; 5 = slightly tender; 8 = extremely tender.
[3]1 = Extremely dry; 4 = slightly dry; 5 = slightly juicy; 8 = extremely juicy.
[4]1 = Extremely bland; 4 = slightly bland; 5 = slightly intense; 8 = extremely intense.

type. There was an association ($P < 0.01$) of the *CAST* marker with juiciness in the GPE7 population. There was an unclear pattern because animals inheriting the CT genotype produced less juicy steaks than animals homozygous for either CC or TT genotypes. An association was observed ($P < 0.01$) between *CAST* and flavor in the GPE8 population. Animals inheriting the CC genotype produced blander steaks than animals inheriting the CT and TT genotypes.

CAPN1 Effect

Levels of significance, least squares means, and standard errors are reported in Table 4 for the effect of *CAPN1* on tenderness score, juiciness, and flavor in the populations studied. For tenderness score, animals inheriting the CC and the CT genotypes produced more tender meat ($P < 0.05$) when compared with animals inheriting the TT genotype in the 3 populations, with the exception that no differences were observed in the STARS population. A significant association was observed for flavor in the GPE8 population, where animals with the CC and the CT genotypes produced steaks with a more intense flavor when compared with animals with the TT genotype.

Interactions

The only significant interaction ($P < 0.05$) between *CAST* and *CAPN1* was observed for WBSF in the GPE8 population (Figure 1). Animals inheriting the CC genotype at both markers were more tender than any other group. Animals inheriting the CC genotype for *CAST* produced tougher meat when they inherited either the CT or the TT genotypes in *CAPN1*.

DISCUSSION

The primary objective of this study was to evaluate a potential genetic interaction between markers for 2 loci that are currently being used as the basis of commercial DNA tests for meat tenderness in beef cattle. There is a strong biological basis for a hypothesis of genetic interaction, as the 2 loci produce proteins that physically interact in determining tenderness. The *CAPN1* locus produces a protease (*CAPN1*) that breaks down myofibrillar protein postmortem, and the *CAST* locus produces an inhibitor (*CAST*) of that protease. The hypothesis is that the effect on tenderness of an allele at one locus may depend on the allele at the other locus, as variation that influences the ability of *CAST* to inhibit *CAPN1* could depend on the physical state or concentration of the enzyme. To test the hypothesis in the animal sets available at the U.S. Meat Animal Research Center, it was necessary to first establish that the markers had individual genetic effects in the GPE7, GPE8, and STARS populations.

Previous studies have suggested that genetic variation at the *CAST* locus contributes to variation in meat tenderness traits (Barendse et al., 2002), but the data presented here represent the first report in the scientific literature of the association of the *CAST* SNP with meat tenderness. (The original finding was patented in 2002.) In the original work, animals homozygous for the T allele were observed to produce meat with lower average shear force than animals homozygous for the C allele. A similar result was obtained in the present study for the 2 populations incorporating *Bos taurus* germplasm. It is possible that the lack of statistical significance in STARS reflects that the present marker system is not adequately matched to functional alleles to be useful in *Bos indicus*

Table 4. Genotype contrasts for tenderness score, juiciness, and flavor intensity of the steak with the marker at the μ-*calpain* (*CAPN1*) gene in the *Bos taurus* (GPE7), *Bos taurus* with *Bos indicus* influence (GPE8), and *Bos indicus* (STARS) populations[1]

		CAPN[2]		
Trait	Population	CC-TT	CT-TT	*P*-value
Tenderness[3]	GPE7	0.26 ± 0.10	0.10 ± 0.09	0.027
	GPE8	0.31 ± 0.14	0.24 ± 0.07	<0.001
	STARS	—	0.07 ± 0.08	0.385
Juiciness[4]	GPE7	0.58 ± 0.07	0.005 ± 0.36	0.244
	GPE8	0.05 ± 0.03	0.06 ± 0.03	0.107
	STARS	—	–0.05 ± 0.07	0.488
Flavor[5]	GPE7	0.22 ± 0.04	–0.04 ± 0.04	0.543
	GPE8	0.10 ± 0.04	0.06 ± 0.03	0.019
	STARS	—	–0.01 ± 0.04	0.844

[1]Values for Warner-Bratzler shear force at d 14 postmortem were previously reported by White et al. (2005).
[2]Mean ± SEM.
[3]1 = Extremely tough; 4 = slightly tough; 5 = slightly tender; 8 = extremely tender.
[4]1 = Extremely dry; 4 = slightly dry; 5 = slightly juicy; 8 = extremely juicy.
[5]1 = Extremely bland; 4 = slightly bland; 5 = slightly intense; 8 = extremely intense.

populations. Alternatively, the influence of the variation may be smaller in the STARS genetic background and fall below a detectable level. In total, the present data support the conclusion that the SNP at the *CAST* gene are associated with functional alleles of *CAST* that affect shear force and indicate that these effects extend to many, but perhaps not all, beef breeds.

A previous study documented the effect of *CAPN1* genotype on shear force in GPE7, GPE8, and STARS; animals homozygous for the C allele had lower average shear force than animals of TT genotype (White et al., 2005). The magnitude of the observed effect on shear force is approximately the same as observed for alleles at *CAST*. The present study also examined tenderness as measured by an expert taste panel. Significant associations of genotype and taste panel tenderness were observed for GPE7 and GPE8, and the magnitude of effect was nearly identical for both markers. In contrast, the reduced tenderness of the unfavorable genotype in STARS did not reach significance because of the lack of CC homozygotes in STARS, which reduced the ability to detect significant association. Because of this constraint in the STARS data, we concluded that the data extend association of genotype at the *CAPN1* SNP to include tenderness as measured by an expert taste panel.

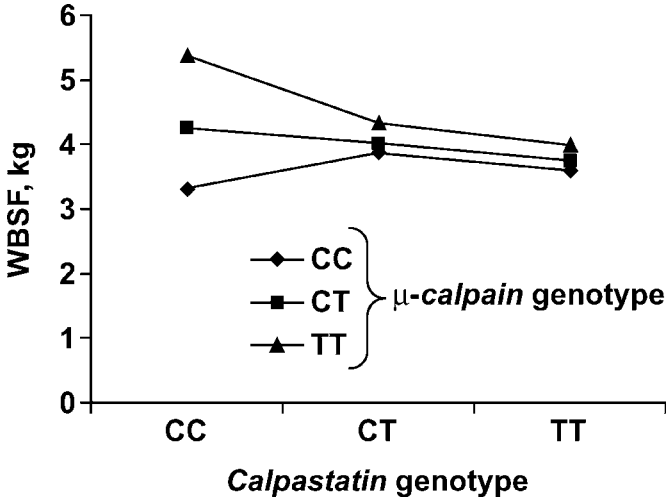

Figure 1. Interaction of μ-*calpain* genotype and *calpastatin* genotype for Warner-Bratzler shear force (WBSF; kg).

The association of both *CAST* and *CAPN1* markers with tenderness in 2 of the 3 populations of widely varied breed makeup supports the investigation of potential genetic interaction between the 2 loci. This analysis depends on the frequency of 2-marker genotypes to compare individual "cells" of genotype class. Allele frequency of the 2 markers in the 3 sets of animals was quite variable. Favorable T allele frequency for the *CAST* SNP was much higher in GPE7 (80%), GPE8 (83%), and STARS (72%) than the favorable C allele of *CAPN1* (58, 64, and 10%, respectively). As a result, some of the 2-genotype cells had very few or no individuals, reducing the power to detect interaction between the 2 loci. Specifically, there was severe under-representation of animals that were homozygous for unfavorable alleles at both markers (CC at *CAST* and TT at *CAPN1*), an important class for detecting interaction in all 3 groups of animals. Nevertheless, the data from GPE7 and GPE8 provide an opportunity to investigate the potential for interaction among most of the possible genotypes.

Analysis of the 2 markers independently suggests that if the loci do not display genetic interaction and act in an additive fashion, the 2-marker genotype with lowest shear force would tend to be TT at *CAST* and CC at *CAPN1*. An apparent interaction was manifest in GPE8 because animals with CC genotype at both markers had the lowest average shear force and the highest tender-

ness rating. It must be emphasized that this genotype cell had only 4 animals; therefore, the mean effect of this combination of genotypes is underestimated, and the result should be considered inconclusive. A more stringent test for the specific effect of the rare 2-marker genotype class would require identification of a population or populations segregating a higher frequency of respective homozygotes at the 2 SNP. The failure to detect interaction in GPE7, in which there were more than twice as many animals in the same genotype cell than in GPE8, further weakens the hypothesis of interaction and suggests that despite the physical interaction of the gene products within the cell, the alleles of the *CAST* and *CAPN1* loci defined by the SNP used in this study probably do not have significant genetic interaction in determining shear force. Therefore, the markers appear to act in an additive fashion in predicting changes in mean shear force in cattle populations.

Data on 2 other measures of meat quality were also evaluated in this study to provide some insight into the possibility that selection based on genotype at these markers might have unintended consequences on other traits. There was no clear evidence for detrimental effects of SNP alleles on juiciness or flavor. There was a statistically significant but small decrease in juiciness among the GPE7 steers with the favorable TT genotype at *CAST* and an increase in flavor of this genotype in GPE8, but because these effects were small and not consistently observed among populations, it is unlikely that they would result in a major impact on phenotype during marker-assisted selection. Similarly, the only significant effect of *CAPN1* genotype was an increase in flavor in the favorable CC genotype in GPE8 animals. The only change in mean with a magnitude >0.23 units for flavor or juiciness in any genotypic class was a statistically nonsignificant increase in juiciness in GPE7 steers with the favorable CC genotype at *CAPN1.* These results suggest that selection on genotype at these 2 loci will have negligible effects on these 2 meat quality traits. We conclude that selection for favorable alleles at *CAST* and *CAPN1* as defined by the SNP genotypes described so far would be likely to improve mean shear force values without genetic interaction between the 2 loci to complicate selection procedures and without discernable effect on meat quality parameters of juiciness and flavor.

IMPLICATIONS

Markers at the bovine *calpastatin* and μ-*calpain* loci that were previously described have been associated with meat tenderness in cattle. This report is the first evaluation of the *calpastatin* marker and demonstrates that the effects of the 2 loci, as identified by the single nucleotide polymorphism used in the study, appear to act in additive fashion to influence shear force. The impact on tenderness and apparent lack of interaction were observed in a wide range of beef breeds including animals of *Bos taurus, Bos taurus,* and *Bos indicus* influence and *Bos indicus* descent. There was no indication that selection for the single nucleotide polymorphism genotype at either locus would have a significant influence on other meat quality traits such as juiciness or flavor.

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