A new single nucleotide polymorphism in *CAPN1* extends the current tenderness marker test to include cattle of *Bos indicus, Bos taurus,* and crossbred descent[1]

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ABSTRACT: The three objectives of this study were to 1) test for the existence of beef tenderness markers in the *CAPN1* gene segregating in Brahman cattle; 2) test existing *CAPN1* tenderness markers in indicus-influenced crossbred cattle; and 3) produce a revised marker system for use in cattle of all subspecies backgrounds. Previously, two SNP in the *CAPN1* gene have been described that could be used to guide selection in *Bos taurus* cattle (designated Markers 316 and 530), but neither marker segregates at high frequency in Brahman cattle. In this study, we examined three additional SNP in *CAPN1* to determine whether variation in this gene could be associated with tenderness in a large, multisire American Brahman population. One marker (termed 4751) was associated with shear force on postmortem d 7 ($P < 0.01$), 14 ($P = 0.015$), and 21 ($P < 0.001$) in this population, demonstrating that genetic variation important for tenderness segregates in *Bos indicus* cattle at or near *CAPN1.* Marker 4751 also was associated with shear force ($P < 0.01$) in the same large, multisire population of cattle of strictly *Bos taurus* descent that was used to develop the previously reported SNP (referred to as the Germplasm Evaluation [GPE] Cycle 7 population), indicating the possibility that one marker could have wide applicability in cattle of all subspecies backgrounds. To test this hypothesis, Marker 4751 was tested in a third large, multisire cattle population of crossbred subspecies descent (including sire breeds of Brangus, Beefmaster, Bonsmara, Romosinuano, Hereford, and Angus referred to as the GPE Cycle 8 population). The highly significant association of Marker 4751 with shear force in this population ($P < 0.001$) confirms the usefulness of Marker 4751 in cattle of all subspecies backgrounds, including *Bos taurus, Bos indicus,* and crossbred descent. This wide applicability adds substantial value over previously released Markers 316 and 530. However, Marker 316, which had previously been shown to be associated with tenderness in the GPE Cycle 7 population, also was highly associated with shear force in the GPE Cycle 8 animals ($P < 0.001$). Thus, Marker 316 may continue to be useful in a variety of populations with a high percentage of *Bos taurus* backgrounds. An optimal marker strategy for *CAPN1* in many cases will be to use both Markers 316 and 4751.

Key Words: Calpain, *CAPN1*, Cattle, Genetic Markers, Meat Tenderness, Shear Force

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Introduction

Beef tenderness is a critical component of palatability, but the difficulty in obtaining phenotypic data until after harvest has made it hard to select for this trait. Therefore, selection for genetic improvement in tenderness has rarely been attempted. Marker-assisted selection could bypass this obstacle if appropriate markers could be found.

Recently, markers in *CAPN1* have been suggested to fill this role (Page et al., 2002; Page et al., 2004). The *CAPN1* gene encodes the protease μ-calpain, which degrades myofibrillar proteins postmortem and is thought to be one of the most important enzymes involved in beef tenderness (Koohmaraie, 1996). The human *CAPN1* gene has 22 exons spanning approximately 30 kb, and bovine *CAPN1* has a similar structure (Figure 1). Page et al. (2002) found two nonsynonymous SNP

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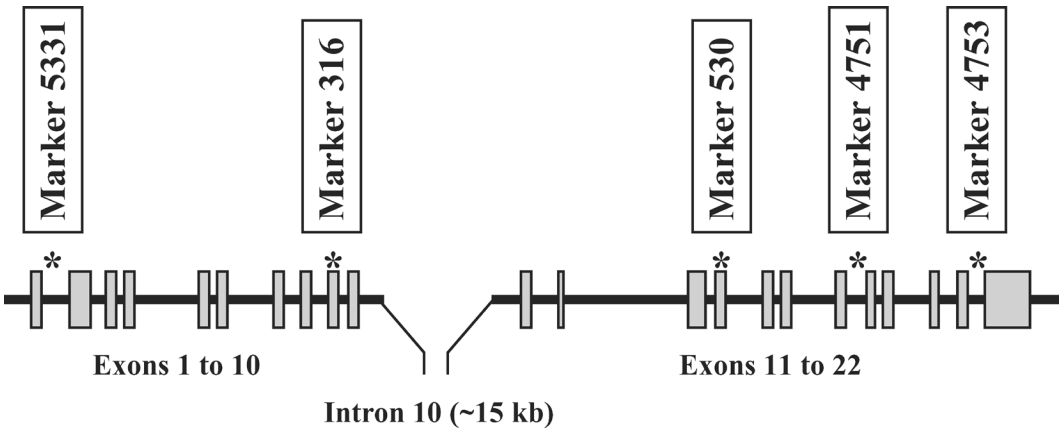

Figure 1. Genomic locations of SNP markers in *CAPN1* gene.

in *CAPN1* that produce AA substitutions at positions 316 (Glycine/Alanine) and 530 (Valine/Isoleucine) in the protein. These SNP consistently identified favorable alleles at tenderness QTL in two distinct resource families (Casas et al., 2000; Morris et al., 2001), and were later shown to be associated with tenderness in a wide range of crossbred *Bos taurus* cattle (Page et al., 2004). Nonetheless, these markers do not segregate at appreciable frequencies in Brahman cattle (Casas et al., 2005). These markers also are homozygous in a Brahman × Hereford bull, which nonetheless segregated a tenderness QTL on BTA 29 (Casas et al., 2003), indicating that Markers 316 and 530 do not identify all variation at *CAPN1* affecting tenderness.

Brahman cattle and their crossbreds are widely used because of their heat tolerance and adaptability. The reputation for less tender beef in these cattle presents an opportunity for improvement by use of genetic markers. The objectives of this study were to develop markers in *CAPN1* that do segregate in cattle of *Bos indicus* descent and to test for the existence of genetic variation at this locus with importance for tenderness.

Materials and Methods

Populations and Phenotypes

Four independent populations were studied. The first population was purebred Brahman, the second was a population of *Bos taurus,* and the third was a population that included germplasm from *Bos taurus* and *Bos indicus.* The fourth population was a QTL resource family segregating a tenderness QTL near *CAPN1* on BTA 29 (Casas et al., 2003). The following is a description of each population.

A population of 504 Brahman calves managed by the SubTropical Agricultural Research Station has been previously described (Riley et al., 2002) and will be referred to herein as the STARS population. Briefly, 22 sires were used over 5 yr to produce 504 Brahman calves in 1996 to 2000 (246 steers, 258 heifers). Calves were fed on site and slaughtered at a commercial facility in Florida. Phenotypic data included Warner-Bratzler shear force (**WBSF**), which is a measure of the force required to pass a blunt blade through a core sample of cooked meat perpendicular to the muscle fibers. Warner-Bratzler shear force data were collected on LM samples from 481 animals at d 7, 14, and 21 postmortem (Riley et al., 2003).

Cycle 7 of the Germplasm Evaluation (**GPE**) project included 554 crossbred steers of *Bos taurus* descent, which 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 seven 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, ¼ Red Poll) cows. Management of cattle and collection of phenotypic data has been recently described by Wheeler et al. (2005). Warner-Bratzler shear force data were collected on LM samples from steers (representing 146/149 sires) at d 14 postmortem (Wheeler et al., 2005).

Cycle 8 of the Germplasm Evaluation project included 597 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 for intercycle GPE standardization purposes. All dams were Angus or MARCIII cows. Management of these animals and collection of phenotypes were similar to GPE Cycle 7 (T. L. Wheeler, personal communication). Warner-Bratzler shear force data were collected on LM samples from steers (representing 125 sires) at d 14 postmortem.

A QTL resource family founded by a Brahman × Hereford F_1 bull had been found to be segregating a tenderness QTL on BTA 29 (Casas et al., 2003). Briefly, 438

Table 1. Genotyping primers for *CAPN1* markers

Marker	Forward primer[a]	Reverse primer[a]	Probe
316	GAGCTGGCCCTCATAAGATAA	CCCATCCTCCATCTTGACC	CCTCGGAGTGGAACG
530	GTGACTTTGTGCTGCGTTTCT	CCTTGCTGGCTAGAGACCAA	GGGGAGATTGGCCTGGA
4751	AAGGGACAGATGTGGACAGG	GAGGGGTGTTCTCTGAGTGC	ACACAGCCCTGCGCCTC

[a]Amplification primers are shown minus standard mass tag.

calves were analyzed, which were sired by the Brahman × Hereford bull and out of Angus, Hereford, and MARC III dams. Management was as previously described (Casas et al., 2003). Warner-Bratzler shear force data were collected on LM samples from all offspring at d 14 postmortem.

Markers and Genotyping

Markers 316 and 530 in *CAPN1* have been previously described (Page et al., 2002). New assays were developed for these SNP to facilitate multiplex genotyping on a MassArray system using matrix-assisted laser desorption/ionization time-of-flight (**MALDI-TOF**) mass spectrometry (Sequenom, Inc., San Diego, CA). Table 1 shows assay primers, including amplification primers, minus a mass tag sequence added by the MassArray system software. Three additional SNP were chosen from the pool generated by Page et al. (2002) that were 1) known to be segregating in the Brahman population; and 2) heterozygous in the Brahman × Hereford bull. These SNP included Markers 5331 and 4753, which have been recently published (Casas et al., 2005), as well as Marker 4751, which is equivalent to position 6545 (C/T) of GenBank Accession No. AF248054 (see Figure 1). Marker names for these markers were derived from U.S. Meat Animal Research Center primer numbers, and have no meaning in regard to *CAPN1* sequence at either the DNA or protein levels. A new MALDI-TOF assay was designed for multiplex genotyping of Marker 4751 with Marker 4753, and Table 1 shows the additional assay primers. The 96-animal U.S. Meat Animal Research Center beef cattle diversity panel version 2.1 (Heaton et al., 2001) was used to screen SNP for segregation in common beef breeds before genotyping on populations with shear force phenotypes. As a quality control measure for genotyping results, all markers were required to comply with Hardy-Weinberg proportions in each multisire population before inclusion in statistical analyses.

Statistical Analyses

Individual association of each marker with WBSF was examined by ANOVA. The GPE Cycle 7 population was analyzed using the Mixed procedure of SAS (SAS Inst., Inc., Cary NC) with shear force as the dependent variable. Sire breed, dam breed, sire breed × dam breed interaction, birth year, slaughter group within year, and marker genotype were included in the model as fixed effects, and weaning age was included as a linear covariate. Sire was included in the model as a random effect nested within sire breed. A similar model was used to analyze the GPE Cycle 8 population. For the Brahman population, the model included contemporary group (44 contemporary groups), sire as a random effect, and the fixed effect of the marker (Casas et al., 2005). For marker 4751, only four individuals homozygous for the "C" allele were observed, and these were dropped for association analysis due to the small sample size. The Brahman × Hereford bull family was analyzed with a model that included effects of gender (heifer or steer), year of birth (1994 or 1996), dam line within year of birth, and days on feed as a covariate.

Markers 316 and 4751 were analyzed simultaneously in both GPE Cycle 7 and GPE Cycle 8. As with the single marker analyses, the model included fixed effects of sire breed, dam breed, sire breed × dam breed interaction, birth year, and slaughter group within year. Weaning age was included as a linear covariate, and sire was included as a random effect nested within sire breed. For each population, a repeat analysis was done that included an interaction between Markers 316 and 4751, so that genotype class differences could be estimated.

Haplotyping was performed using PHASE v2 (Stephens et al., 2001; Stephens and Donnelly 2003). Most likely haplotypes were included in the analysis if assigned with probabilities of 90% or more. In the GPE Cycle 7 population, sire genotypes were available for 131 sires, and steer haplotypes were discarded if non-Mendelian inheritance was observed (i.e., if sires and sons were alternate homozygotes). In both the GPE Cycle 7 and GPE Cycle 8 populations, haplotypes comprising Markers 316, 530, and 4751 were compared against the GAT haplotype shown to be associated with the less tender phenotype by a previous study (Page et al. 2004). The null hypothesis in each case was no effect of haplotype, and the additive effect of each haplotype was estimated assuming no dominance interaction. Because there were six haplotypes, this test had 5 df (subsequently referred to as the 5-df test).

Results

The starting point for the study was the *CAPN1* gene sequence data on the two alleles of a Brahman × Hereford bull that had been used to sire a large resource population with evidence of a tenderness QTL on BTA

Table 2. Genotype contrasts for shear force at d 14 postmortem with marker 4751 in the purebred Brahman, Germplasm Evaluation (GPE) Cycle 7 and Cycle 8 populations

	Population					
	STARS Brahmans		GPE Cycle 7		GPE Cycle 8	
Genotypes	WBSF, kg[a]	No.	WBSF, kg[a]	No.	WBSF, kg[a]	No.
CC	—	0	–0.27 ± 0.10	187	–0.44 ± 0.10	237
CT	–0.40 ± 0.15	91	0.014 ± 0.093	260	–0.28 ± 0.095	289
TT	0.00	380	0.00	103	0.00	70
P-value	$P = 0.015$		$P = 0.003$		$P < 0.001$	

[a]Deviation in Warner-Bratzler shear force (WBSF).

29 encompassing the map position of bovine *CAPN1*. Sequence data on this bull indicated that previously described Markers 316 and 530 were homozygous (fixed for the G alleles at both loci). It was expected that polymorphisms within the QTL region would show a similar association with WBSF to that revealed by the interval mapping study based on microsatellite genotypes (Casas et al., 2003). Indeed, two *CAPN1* polymorphisms (Markers 4751 and 4753, lying in introns 17 and 21, respectively; Figure 1) segregating in this sire's offspring were genotyped in the resource calves and confirmed association with shear force (both $P < 0.01$). A third SNP (Marker 5331) was not run in this family because the other markers had already established a relationship between *CAPN1* and the QTL for shear force.

The SNP were identified in a crossbred bull, so it was possible that they would be uninformative within either purebred Brahman or strictly *Bos taurus* animals. To test SNP informativity and utility within Brahman cattle, Markers 4751, 4753, and 5331 were genotyped in the STARS Brahman population. Marker 4751 segregated with a rare allele (C) frequency of 10.8%, and showed association ($P = 0.015$) with d-14 shear force (Table 2). Marker 4751 also was associated with shear force at postmortem d 7 ($P < 0.01$) and 21 ($P < 0.001$) in this population. The genotype contrasts (TT – CT) were 0.51 ± 0.15 kg for d-7 shear force, 0.40 ± 0.15 kg for d-14 shear force, and 0.52 ± 0.14 kg for d-21 shear force, with CT as the lower shear force genotype in each case. A haplotype analysis including Markers 316 and 530 was not performed for the STARS population because these markers were almost fixed for G at both loci in this population (Casas et al., 2005), and therefore would not add significant information to the individual 4751 marker analysis. A haplotype analysis in the STARS Brahmans was attempted on three-marker haplotypes, including Markers 5331, 4751, and 4753, but this also failed to add information to the individual analysis of Marker 4751 (data not shown).

Although Markers 316 and 530 have been shown to be useful in the strictly *Bos taurus* GPE Cycle 7 population, it was of interest to determine whether Marker 4751 was segregating in these animals and whether it also would show association with tenderness. In GPE Cycle 7, Marker 4751 allele C segregated with a frequency of 57.5%, and showed highly significant association with shear force at d 14 ($P = 0.003$; Table 2). Markers 5331 and 4753 were not segregating at appreciable frequencies in initial samples of *Bos taurus* cattle, and so were not tested in GPE Cycle 7. A two-marker analysis that fit Markers 316 and 4751 simultaneously (see Table 4) showed that 4751 continued to be significant ($P = 0.01$), but 316 did not ($P = 0.21$). Four haplotypes were found to segregate at greater than 1% frequency in the GPE Cycle 7 population: CGC, GGC, GGT, and GAT, where each letter indicates the genotype of Marker 316, 530, and 4751, respectively. Genotypes consisting of these haplotypes showed significant association with shear force ($P = 0.03$), and haplotypes CGC and GGC were associated with lower shear force than GGT and GAT (see Table 5).

Further testing of the applicability of Marker 4751 among breeds was provided by the GPE Cycle 8 population, which includes *Bos indicus*-influenced crossbreds with Brangus, Beefmaster, and Bonsmara sire breeds. Marker 4751 allele C segregated at a frequency of 63.9% in GPE Cycle 8, and there was a highly significant association with shear force at d 14 in this population ($P < 0.001$; Table 2). Marker 5331 also was tested in GPE Cycle 8, where allele A segregated at 7.3% frequency, but as in the STARS population, it did not show association with shear force (data not shown). Marker 4753 allele A segregated at 6.1% frequency and showed association with shear force ($P = 0.041$). The GPE Cycle 8 population also was genotyped with Markers 316 and 530, as preliminary studies (not shown) indicated that they segregated in these families. The Marker 316 allele C segregated at 21.9% frequency in GPE Cycle 8, and was associated with shear force in GPE Cycle 8 ($P < 0.001$; Table 3). Marker 530 allele A segregated at 14.0% frequency, but it did not show significant association with shear force in the GPE Cycle 8 population ($P = 0.076$; Table 3). A two-marker analysis that fit Markers 316 and 4751 simultaneously (Table 4) showed that both were highly significant ($P < 0.01$). The same four haplotypes composed of Markers 316, 530, and 4751 observed in GPE Cycle 7 were found to segregate at greater than 1% frequency in GPE Cycle 8, and a significant effect of haplotype on shear force was identified

Table 3. Genotype contrasts for shear force at d 14 postmortem with *CAPN1* Markers 316 and 530 in Germplasm Evaluation Cycle 8

Genotypes	WBSF, kg[a]	No.	*P*-value
Marker 316			
CC	–0.55 ± 0.14	31	$P < 0.001$
CG	–0.18 ± 0.061	221	
GG	0.00	347	
Marker 530			
AA	0.39 ± 0.20	12	$P = 0.076$
AG	0.094 ± 0.068	141	
GG	0.00	447	

[a]Deviation in Warner-Bratzler shear force (WBSF).

($P < 0.01$). Again, haplotypes CGC and GGC were associated with lower shear force than GGT and GAT (Table 5).

Discussion

Marker-assisted selection has great potential to improve traits for which selection has been historically difficult, such as meat tenderness. The current challenge is to find markers for such traits that will be useful in many populations, especially those with the greatest opportunity for improvement in those traits. The majority of DNA markers developed for quantitative traits in the next few years is not likely to be the causative nucleotide variation because identification and proof of nucleotide substitutions with low to moderate effects is extremely difficult. However, markers that effectively track functional alleles in a reliable way have substantial value because they permit relatively accurate assessment of genetic merit at a locus based solely on the genotype of the individual animal, without the need for extensive testing of pedigree material to determine the presence of variation and phase with respect to marker alleles. Examples of this type of marker are the SNP Markers 316 and 530 previously shown to have predictive merit in both the GPE Cycle 7 population and a commercial sample of Simmental × Angus calves (Page et al., 2004). Neither marker is likely to represent the nucleotide difference causing the influence on the trait, but the data so far indicate that they are useful for tracking functional alleles with respect to tenderness in major *Bos taurus* beef cattle breeds.

Cattle of *Bos indicus* descent are widely used for their heat tolerance and disease resistance, but tenderness has been problematic in many of these animals (Crouse et al., 1989). Because previously released *CAPN1* Markers 316 and 530 are almost fixed in *Bos indicus* cattle (Casas et al. 2005), the initial goal of this study was to provide markers segregating in *Bos indicus* cattle associated with effects on meat tenderness. The first part of the discussion below focuses on the development of Marker 4751 and assessment of its predictive merit in populations of *Bos indicus, Bos taurus,* or *Bos indicus* × *Bos taurus* crossbred cattle. The second section compares these results with those of the other *CAPN1* markers in these populations. The final section addresses haplotypes and combinations of markers likely to be useful in making selection decisions.

CAPN1 Marker 4751

The initial goal of this study was to identify *CAPN1* markers tracking functional alleles affecting tenderness in *Bos indicus* cattle. Marker 4751 is significantly associated with WBSF at d 14 postmortem in a large, multisire population of American Brahman cattle. The difference between the favorable CT heterozygotes and the TT homozygotes was estimated to be 0.4 kg of shear force. The additional shear force data collected on these animals at d 7 and 21 postmortem confirm this association, with even larger estimated differences of 0.5 kg of shear force between CT heterozygotes and TT homozygotes, and highly significant associations at both time points. Together, these results suggest that *CAPN1*

Table 4. Genotype contrasts for shear force at d 14 postmortem with both *CAPN1* Markers 316 and 4751 fit simultaneously in the Germplasm Evaluation (GPE) Cycle 7 and Cycle 8 populations

	Marker 4751 genotype					
	CC		CT		TT	
Marker 316 genotype	WBSF, kg[a]	No.	WBSF, kg[a]	No.	WBSF, kg[a]	No.
GPE Cycle 7 population						
CC	–0.56 ± 0.24	17	–0.03 ± 0.46	3	—	0
CG	–0.38 ± 0.13	82	–0.21 ± 0.12	82	–0.37 ± 0.33	6
GG	–0.31 ± 0.12	86	–0.06 ± 0.10	162	0.00	94
GPE Cycle 8 population						
CC	–0.80 ± 0.16	29	–1.22 ± 0.47	2	—	0
CG	–0.50 ± 0.12	98	–0.41 ± 0.11	112	–0.01 ± 0.27	7
GG	–0.37 ± 0.12	105	–0.25 ± 0.10	172	0.00	61

[a]Deviation in Warner-Bratzler shear force (WBSF).

Table 5. Genotype contrasts for shear force at d 14 postmortem with haplotypes of *CAPN1* Markers 316, 530, and 4751 in the 5-df test of the Germplasm Evaluation (GPE) Cycle 7 and Cycle 8 populations

	GPE Cycle 7 population		GPE Cycle 8 population		Overall
Haplotype	WBSF, kg[a]	No.	WBSF, kg[a]	No.	frequency
CGC	–0.26 ± 0.08	209	–0.31 ± 0.07	266	21.2%
GGC	–0.13 ± 0.07	399	–0.14 ± 0.06	495	39.9%
GGT	–0.02 ± 0.08	153	0.03 ± 0.07	260	18.4%
GAT	0.00	283	0.00	160	19.8%
CAT	–0.11 ± 0.34	6	–0.14 ± 0.50	2	0.36%
CGT	0.23 ± 0.55	2	–0.15 ± 0.27	7	0.40%
P-values	$P = 0.03$		$P < 0.001$		

[a]Deviation in Warner-Bratzler shear force (WBSF).

Marker 4751 may be an excellent marker for functional variation affecting tenderness in Brahman cattle, which satisfies the initial project objective.

Because Marker 4751 also segregates in *Bos taurus* cattle in addition to the initial target *Bos indicus* population, we also tested Marker 4751 in the GPE Cycle 7 population. This purely *Bos taurus* population was designed to represent diverse germplasm from the seven most populous beef breeds in the United States by annual registrations. The multisire, multibreed structure of the GPE Cycle 7 population makes it a rigorous test for association of any of the recorded traits with genetic markers. Notably, Marker 4751 showed highly significant association with shear force in this population (Table 2). Nineteen of 22 Angus sires of GPE Cycle 7 were successfully genotyped, and these had a higher frequency of the favorable C allele of Marker 4751 (84.2%) compared with sires from the other breeds (40.6 ± 21.7%). This finding is a result similar to that observed for these sires with marker 316 (Page et al., 2004), and it raises the potential for population stratification artifacts in the association analysis if Angus sires tend to be at the favorable extreme of the phenotype distribution. However, the model used included adjustments for sire breed, and removal of the Angus-sired individuals from the analysis does not eliminate the highly significant association of Marker 4751 with shear force in this multi-breed population (data not shown). From the Marker 4751 study in GPE Cycle 7, it is crucial to note that the phase of favorable association is the same in both the Brahman population and the GPE Cycle 7 population, with "C" as the favorable allele. This suggests that Marker 4751 may be useful in many populations, including those of *Bos indicus*-influenced crossbred descent.

To further examine this hypothesis, we tested Marker 4751 in an additional population with many *Bos indicus*-influenced crossbred individuals. The GPE Cycle 8 population was designed to assess several tropically adapted breeds, including some *Bos indicus*-influenced breeds. Because it has the same broad germplasm sampling pattern as GPE Cycle 7, the GPE Cycle 8 population also is a very stringent test for marker-trait association. Marker 4751 was significantly associated with shear force in GPE Cycle 8, again with "C" as the favorable allele. The extremely low *P*-value ($P < 0.001$) confirms the usefulness of Marker 4751 for predicting tenderness, even in a *Bos indicus*-influenced crossbred population. This finding is interesting in light of the possibility that different causative mutations could be segregating in the two subspecies of cattle. If so, it is less likely that a single marker would be in phase with the different favorable alleles in both populations, although such association is possible by chance. An alternative interpretation of the data is that there is an identical causative mutation present in both subspecies, but the 4751 polymorphism occurred before the split between *Bos indicus* and *Bos taurus* subspecies, and the 316 marker polymorphism occurred later and specifically in the *Bos taurus* lineage. Regardless, the consistent phase association is important because it implies that Marker 4751 may be widely useful to predict variation in meat tenderness in cattle of both subspecies, as well as crossbreds.

Comparison of Marker 4751 with Other CAPN1 Markers Tested

Besides Marker 4751, additional *CAPN1* markers were shown to have significant associations with shear force in these populations. Most notably, whereas *CAPN1* Marker 316 does not segregate at appreciable frequencies in the STARS Brahmans, it does segregate and show highly significant association with shear force in GPE Cycle 8. The extremely low *P*-value supports the usefulness of this marker, even in a population with some *Bos indicus* influence. This broadens the range of demonstrated usefulness of this commercially available marker beyond the results reported to date in strictly *Bos taurus* cattle.

Another *CAPN1* marker, 4753, also shows significant association with shear force in GPE Cycle 8; however, the association is not as highly significant as for Markers 4751 and 316, and it is not repeated in other populations (for Markers 4753 and 5331 in STARS Brahmans, see Casas et al., 2005). Occasional associations of this

type can be expected in a region near a functional mutation, although repeatability in many populations is clearly a key for determining which genetic markers are of greatest commercial value.

Finally, Marker 530 also was tested in GPE Cycle 8, but it had only a trend of association in this population (Table 3). Marker 530 previously had been shown to have a highly significant association with shear force in the GPE Cycle 7 population (Page et al., 2004). Together these results indicate that although Marker 530 has an association with tenderness in many cattle breeds, there are populations in which this marker will not be as useful. For this reason, Markers 316 and 4751 should be preferred because they show association with tenderness in a wide variety of populations.

CAPN1 Haplotypes and Useful Marker Combinations

In previous studies (Page et al., 2004), *CAPN1* haplotypes composed of 316–530 genotypes gave four classes of phenotypic effects: CG tender, GG intermediate, GA tough, and CA inestimable (rare). Marker 4751 divides the population in a nearly identical way as these haplotypes, with the C allele of 4751 containing virtually all of the CG and the T allele of 4751 containing all of the GA haplotype animals (Table 5). The higher significance of the association of Marker 4751 probably results from the fact that it is able to subdivide the GG haplotype in such a way as to increase association with shear force in the same GPE Cycle 7 population. The GGT haplotype is almost equivalent in effect to the GAT haplotype (Table 5), indicating that this subset of GG alleles carry few, if any tender causative mutation(s). The GGC haplotype has an effect intermediate to the CGC tender allele and the GGT/GAT tough alleles, indicating that this class of haplotypes might contain a mixture of tender and tough causative mutation(s). If this is the case, the frequency of the causative mutation(s) would be underestimated by the frequency of marker 316 (19.8% in GPE Cycle 7; 21.9% in GPE Cycle 8) but overestimated by the frequency of Marker 4751 (57.5% in GPE Cycle 7; 63.9% in GPE Cycle 8), at least in *Bos taurus*-derived cattle. Thus, it should be possible to improve on both Marker 316 and Marker 4751 by finding SNP that better subdivide the GGC haplotype to achieve tighter population-wide linkage disequilibrium with causative mutation(s).

A central question is which marker or markers will be the most useful in a given population. Given the haplotype analyses, Markers 316 and 4751 have the best support for usefulness in many situations. Of these, Marker 4751 is the only one likely to be useful in populations with a high percentage of *Bos indicus* influence because the favorable allele of Marker 316 will likely be rare in such populations. Additionally, a simultaneous analysis of Markers 316 and 4751 in the strictly *Bos taurus* GPE Cycle 7 population suggests that there are situations in which marker 316 will fail to explain additional variation in tenderness once Marker 4751 is taken into account. However, a simultaneous analysis of Markers 316 and 4751 in GPE Cycle 8 shows that both markers have highly significant effects, even in a *Bos indicus*-influenced population. Furthermore, the haplotype data in Table 5 suggest that the lowest shear force is associated with the haplotype containing favorable alleles at both markers, which further supports the usefulness of both. A multiplex genotyping assay incorporating both markers retains the strengths of both, and may be the most useful approach.

In this study, we have shown that important genetic variation for tenderness segregates in *Bos indicus* cattle near the *CAPN1* gene, and we have provided a useful marker for this variation. The 4751 marker seems to have broad usefulness in cattle of *Bos taurus, Bos indicus,* and crossbred descent. We also have shown that the *CAPN1* 316 marker has strong association with tenderness in a population with historical *Bos taurus*/*Bos indicus* admixture. A multiplex marker system incorporating both Markers 316 and 4751 provides an optimal solution in all populations studied to date.

Implications

Genetic markers for the bovine *CAPN1* gene previously have been published and associated with meat tenderness in cattle of *Bos taurus* descent. The current work supplies an additional marker associated with meat tenderness in Brahman cattle. This new marker is also associated with tenderness in *Bos taurus* cattle, as well as crossbred cattle. The wide applicability of the new marker provides a simple genetic test not restricted to *Bos taurus* cattle. The current work also demonstrates the extended usefulness of a previously published *CAPN1* marker in some crossbred cattle but not in Brahmans. These results expand possibilities for using genetic markers to improve meat tenderness in many commercial herds, especially those including cattle of Brahman and/or crossbred descent.

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