Association of markers in the bovine *CAPN1* gene with meat tenderness in large crossbred populations that sample influential industry sires[1,2]

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ABSTRACT: Two previously identified single-nucleotide polymorphism markers located within the micromolar calcium-activated neutral protease gene (*CAPN1*) were evaluated for their association with variation in meat tenderness using one commercial sample of Simmental × Angus crossbred calves and one multibreed, crossbred research herd. The commercial sample included 362 animals sired by 23 registered Simmental bulls bred to unregistered Angus cows and represented current industry animals in which to test the predictive merit of the markers. The second sample was a research herd including 564 steers from the Germplasm Evaluation Cycle VII population at the U.S. Meat Animal Research Center, produced with semen from popular sires of the seven *Bos taurus* beef breeds with the most registrations in the United States (Angus, Charolais, Gelbvieh, Hereford, Limousin, Red Angus, and Simmental) on Angus, Hereford, and MARC III cows. These animals form a relatively outbred population that constituted a stringent test of the predictive merit of the genetic markers, although small groups were half-sibs. Warner-Bratzler shear force measurements were used to determine tenderness phenotypes for all animals. The populations were genotyped for two markers that predict variation at amino acid positions 316 and 530 of the μ-calpain polypeptide, produced by the *CAPN1* gene. Minor allele frequencies for markers 316 and 530 in the commercial sample were 0.17 and 0.37, respectively, and in the Cycle VII animals, were 0.20 and 0.28, respectively. Both markers showed association with shear force in the commercial sample (P = 0.04) and the Cycle VII population (P = 0.02), supporting the hypothesis that they represent potential markers to aid selection for improved meat tenderness in commercial populations of beef cattle in the United States.

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

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Introduction

Meat tenderness is a critical trait in determining consumer satisfaction, and there has been significant interest in genetic selection to decrease problems with meat tenderness variation. However, the problem of variability in meat tenderness has not diminished, in part because of an inability to accurately select for increased tenderness. Identification of genetic markers for meat tenderness variation would provide some selection criteria to facilitate genetic improvement in this trait.

Previous studies of resource populations produced from Piedmontese × Angus or Jersey × Limousin sires identified a QTL influencing meat tenderness on chromosome 29 (Casas et al., 2000). Subsequently, the bovine calcium-activated neutral protease (***CAPN1***) gene,

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encoding the protease μ-calpain, was mapped to the QTL interval (Smith et al., 2000). This protease seems to be the primary enzyme in postmortem tenderization (Koohmaraie, 1996), suggesting from both positional and functional standpoints that variation in the gene sequence might be associated with meat tenderness in cattle.

Sequencing the bovine *CAPN1* gene in a multibreed panel of cattle identified >150 sequence variations spread along >11,000 bp. Two single-nucleotide polymorphisms (**SNP**) predict variation in the protein sequence of the protease (Page et al., 2002), and both the Piedmontese × Angus and Jersey × Limousin sires of the two resource populations were heterozygous for two isoforms of the protease based on these two AA differences. Due to the nature of QTL populations (i.e., single sire, half-sib family structure), this result was encouraging, but it did not provide compelling evidence that the markers have predictive merit. The objectives of the current study were to test the predictive merit of these markers in a sample of commercial Simmental × Angus beef cattle, to investigate the utility of these markers in a larger, outbred set of animals, and to increase confidence in allele frequency estimates among U.S. beef cattle.

Materials and Methods

Resource Animals and Phenotype Collection

The commercial sample consisted of 362 progeny of 23 registered Simmental sires provided by the American Simmental Association (**ASA**) and hereafter referred to as the ASA sample. Sires had from 4 to 56 progeny; 15 sires had 10 to 26 progeny, one had more, and seven had fewer. Most were part of the Carcass Merit Traits Project sponsored by the National Cattlemen's Beef Association (Dikeman et al., 2003), but a few groups were from the ASA sire progeny-testing program with data collection according to the Carcass Merit Project protocol. The dams, predominately commercial Angus cows, were from eight ranches and were bred by AI. Animals were assigned to contemporary groups based on source, sex, and slaughter date. Consequently, contemporaries were of the same sex, fed in the same feedlot for the same length of time, and harvested at the same processing plant at a similar age (they resulted from a single synchronized mating). Management practices, however, varied among contemporary groups. For the ASA sample, Warner-Bratzler shear force (**WBSF**) measurements, which determine the relative force required to pass a blunt blade through a section of cooked meat, were collected at Kansas State University as described (King et al., 2003) on cooked LM steaks aged 14 d postmortem.

The USDA Meat Animal Research Center (**MARC**) Germplasm Evaluation Cycle VII population is described in detail in a separate manuscript (Wheeler et al., 2004). Briefly, the population included 564 steers generated by AI with semen of bulls from the seven beef breeds with the highest number of registered animals in the United States. Semen from 22 Angus, 22 Charolais, 23 Gelbvieh, 21 Hereford, 20 Limousin, 21 Red Angus, and 20 Simmental sires was used to produce mainly crossbred progeny (except where Angus or Hereford sires were used on cows of the same breed). Ten bulls from each breed were selected to include sires among the top 50 in progeny registrations in their respective herdbooks, with young unproven sires making up the remaining 10 to 13 bulls per breed. The Cycle VII dams were Angus, Hereford, and composite MARC III (¼ Angus, ¼ Hereford, ¼ Pinzgauer, and ¼ Red Poll). Steer calves were born in March through April of 1999 and 2000 (average 3.8 per bull, range 0 to 10; average 80 per sire breed, range 74 to 84; 188, 88, and 269 out of Angus, Hereford, and MARC III dams, respectively). Steers were castrated within 24 h of birth, weaned at 200 d of age, and assigned to pens replicated within sire breed after a postweaning adjustment period of about 30 d. Each pen of animals was fed separately for an average of 239 d, and all steers received 200 mg of progesterone and 20 mg of estradiol benzoate implants in December and again in March. Calves were slaughtered in five groups per year in May and June at commercial facilities, and rib sections were collected for analysis at MARC. Meat tenderness data were collected using WBSF as described (Wheeler et al., 2004) on cooked LM steaks aged 14 d postmortem.

Genotyping

Genotyping of the Cycle VII population 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), as described by Stone et al. (2002). Genotypes for each animal were collected, and the automated calls were checked by manual visualization of the spectrographs to minimize error. Marker-specific primer sequences for genotyping the 316 marker were as follows:

5′-GGGCCAGATGGTGAACCTGA-3′ Forward amplification primer
5′-TTGCGGAACCTCTGGCTCTT-3′ Reverse amplification primer
5′-CAGCTCCTCGGAGTGGAACG-3′ Probe primer

Primers for genotyping the 530 marker were as follows:

5′-GAGCCCAACAAGGAAGGT-3′ Forward amplification primer
5′-AATACAGCCCAATGATGAGG-3′ Reverse amplification primer
5′-GCAGAGAGCTGGATGACCAG-3′ Probe primer

Additional universal primer or mass tag sequence was added to the 5′ end of the amplification primers as

recommended for the particular assay by the MassArray system software.

Genotyping of the ASA commercial population was performed as a service by GeneSeek, Inc. (Lincoln, NE), also using a MassArray system.

Association Analysis

Similar analyses were performed on Cycle VII and ASA shear force data using the Mixed procedure of SAS (SAS Inst., Inc., Cary NC). The model for ASA data included fixed effects for marker genotype(s), as defined below, and contemporary group, plus a random sire effect. Including relationships among sires had little effect on ASA results (unpublished data). To be consistent between analyses, ASA sires were assumed to be unrelated. For the more complex Cycle VII population, contemporary group was replaced in the model with sire breed, dam breed, sire breed × dam breed interaction, birth year, slaughter group, and weaning age as fixed effects. Sires were again assumed unrelated and were included in the model as random effects.

Four analyses were conducted with the dependent variable of marker genotype variously defined. In three analyses marker genotypes were considered to be independent. For the first two analyses, effect of marker genotype was analyzed for each SNP individually (marker 316 was CC, CG, or GG genotype; marker 530 was AA, AG, or GG genotype). Two-marker genotypes of each animal were then considered jointly, with the same model as for individual markers, for the third analysis. The fourth analysis predicted haplotypes of the two markers as dependent variable, given the four possible haplotypes C/A, C/G, G/A, G/G, where the first allele is the 316 marker and the second allele is the 530 marker. The results were estimated by four regressions on expected numbers of each haplotype inherited given the marker genotypes. Because the covariates sum to two, only three contrasts among the regression coefficients are estimable, and the analysis fit the expected number of haplotypes inherited (i.e., 0, 1, or 2 copies of a given haplotype). Animals heterozygous at both markers could not be directly assigned haplotypes, so maximum likelihood estimates of haplotype were assigned based on haplotype frequency computed from two-locus genotypes of purebred sires via expectation maximization algorithm. Haplotype frequencies for Cycle VII animals were computed from frequencies of Cycle VII purebred sires; frequencies for the ASA sample were computed from genotypes of the 36 purebred Simmental sires available to ASA, with frequencies for the commercial dams being assumed similar to the overall Cycle VII estimates. These estimates were considered known in the haplotype analysis. The results are presented as deviations from the mean of the most common (G/G) haplotype. The null hypothesis was no effect of haplotype, and the additive effect of each haplotype was estimated assuming no dominance interaction. Tests of marker effects were performed using the Kenward-Roger method for calculating denominator degrees of freedom (SAS Inst., Inc.).

Table 1. Genotype contrasts for shear force with individual *CAPN1* markers in the American Simmental Association (ASA) and Cycle VII populations.

	ASA population		Cycle VII populations	
Genotypes	WBSF, kg[a]	No.	WBSF, kg[a]	No.
316 marker				
CC	–0.63 ± 0.29	9	–0.29 ± 0.19	22
CG	–0.24 ± 0.10	106	–0.16 ± 0.07	181
GG	0	247	0	349
530 marker				
AA	0.38 ± 0.15	42	0.20 ± 0.13	47
AG	0.04 ± 0.10	181	0.18 ± 0.07	210
GG	0	139	0	295

[a]Deviation in Warner-Bratzler shear force.

Results

Definition of Markers, Alleles, and Haplotypes

Two SNP markers were employed in this study, one of which lies in exon 9 and the other in exon 14 of the bovine *CAPN1* gene (Page et al., 2002); both predict AA sequence changes in the μ-calpain protein. Specifically, a guanine (G allele) to cytosine (C allele) transversion in exon 9 predicted either glycine or alanine at AA number 316 (designated marker 316), and an adenine (A allele) to guanine (G allele) transition in exon 14 predicted either isoleucine or valine at AA number 530 (designated marker 530). Haplotypes depend on the alleles of the two markers on individual chromosomes and are defined by the allele at marker 316, presented first when discussing haplotype, followed by a slash and the allele at marker 530. For example, the haplotype coding for glycine and isoleucine (haplotype G/A at the two markers) was observed in both the Piedmontese × Angus and Jersey × Limousin sires used in the QTL resource populations of the previous study (Page et al., 2002), and was found to be associated with increased shear force relative to the alternative alanine and valine haplotype (haplotype C/G) in both resource populations.

Allele Frequencies in the Test Populations

The genotypes of the crossbred calves were used to determine allele frequency in the two test populations (derived from genotype counts given in Table 1). Genotypes for both markers in all 362 calves of the ASA population were successfully generated. The minor allele frequency of marker 316 (C allele) was 17% and of marker 530 (A allele) was 37% in this population. In the Cycle VII population, 147 of the 149 purebred sires produced steer calves for this study, and 134 sires gave definitive genotypes at both markers. Among Cycle VII steers, 552 of the 564 calves gave definitive genotypes

Table 2. Genotype contrasts for shear force with both *CAPN1* markers fit simultaneously in the American Simmental Association (ASA) and Cycle VII populations

	316 marker genotype					
	CC		CG		GG	
530 marker genotype	WBSF, kg[a]	No.	WBSF, kg[a]	No.	WBSF, kg[a]	No.
ASA population						
AA	ND	0	–0.52 ± 0.42	4	0.25 ± 0.17	38
AG	–0.36 ± 0.50	3	–0.15 ± 0.15	57	0.02 ± 0.13	121
GG	–0.69 ± 0.36	6	–0.23 ± 0.16	45	0	88
Cycle VII population						
AA	ND	0	–0.016 ± 0.37	5	0.18 ± 0.14	42
AG	0.20 ± 0.56	2	–0.01 ± 0.13	50	0.19 ± 0.09	158
GG	–0.24 ± 0.20	20	–0.07 ± 0.10	126	0	149

[a]Deviation in Warner-Bratzler shear force.

at both markers. Minor allele frequencies for the 316 and 530 markers were 20% and 28%, respectively, in the Cycle VII population.

Two-marker genotype frequencies for calves of the two populations (derived from two-marker genotype counts given in Table 2) revealed a difference in frequency between the multibreed crossbred Cycle VII population and the ASA population. Specifically, Cycle VII calves had approximately twice the frequency of animals heterozygous for the 316 marker, and homozygous GG genotype for the 530 marker (23%), compared with the ASA population (12%).

The two-marker genotype data were used to determine two-marker haplotypes, unambiguously for the 807 calves that were homozygous for at least one of the two markers (e.g., if an animal's genotype at the two markers is CC and AG, then it must carry one C/G haplotype and one C/A haplotype). The remaining 107 calves were double heterozygotes and could not be definitively assigned haplotypes because they could result either from a combination of C/G and G/A haplotypes or C/A and G/G haplotypes. However, the haplotypes for these calves could be estimated based on the observed frequency of haplotypes among the sires of each group of animals. For the ASA sample, the estimate was that 13% of double heterozygotes were made up of C/A and G/G haplotypes, and the remaining 87% were C/G and G/A. The estimate in the Cycle VII population was that 10% of double heterozygotes contain the rarer C/A haplotype. The numbers of animals indicated for each haplotype and the resulting haplotype frequencies in Table 3 and 4 applied this estimate to double heterozygote calves.

The genotypes observed in the Cycle VII purebred sires are presented in Table 4 by breed. The relatively small number of bulls of each breed (20 to 23) used to generate the Cycle VII population limits the ability to estimate allele frequency by breed; however, the data suggest that the C allele of marker 316 has a higher frequency in Angus, and the A allele of marker 530 has a lower frequency in this breed ($P < 0.001$). In the Angus purebred sires, the C allele was actually the major allele (59%, Table 4), whereas the C allele frequency in the other purebred sire breeds ranged from 0 to 19%. Two-marker genotype data for the Cycle VII sires (data not shown) were used to determine haplotype counts for each sire breed, applying the estimate that 10% of double heterozygotes carry C/A haplotype alleles. Two C/A haplotype alleles were directly observed among the 134 sires with successful two-marker genotypes, one each in Limousin and Simmental sires, but no breed had more than two double heterozgote individuals. Therefore, no additional C/A haplotypes were predicted among the total of 10 sires with double heterozygote genotypes.

The data in Table 4 show that the higher incidence of C allele at marker 316 in Angus sires is reflected in an increased percentage of animals with C/G haplotype. Angus sires had more than eight times higher percentage of C/G haplotype compared with the average of all other breeds (57 vs. 7 ± 6%), due to the fact that 16 of the 21 Angus sires carried at least one C/G haplotype with four sires homozygous for C/G (data not shown). The decreased frequency of the A allele at marker 530 in Angus sires was reflected in a decreased frequency of G/A haplotype (7% in Angus sires vs. 40 ± 20% for other sire breeds) and the absence of a C/A haplotype allele in Angus sires.

To determine whether the increased C/G haplotype frequency was an artifact of the limited sample of Angus sires, a panel of 192 purebred Angus bulls (described in Heaton et al., 2001) was genotyped for both markers, generating successful two-marker genotypes for 191 animals. The haplotype frequencies of this bull panel are also shown in Table 4. In this group of animals, the expectation maximization algorithm calculation for double heterozygotes indicated 0% predicted to contain C/A haplotype, due to the failure to observe even a single unambiguous C/A haplotype among the 171 animals (242 haplotypes) that could be definitively assigned. Therefore, the results in the bull panel column of Table 4 reflect adjustment of all 21 double heterozygous individuals assigned to C/G plus G/A haplotypes. Comparison of haplotype frequency in the bull panel to

Table 3. Genotype contrasts for shear force with haplotypes of *CAPN1* markers in three degree of freedom test for the American Simmental Association (ASA) and Cycle VII populations

	ASA Population		Cycle VII population		Overall
Haplotype	WBSF, kg[a]	No.	WBSF, kg[a]	No.	frequency
C/A	–0.35 ± 0.31	16	–0.03 ± 0.31	12	0.01
C/G	–0.19 ± 0.11	108	–0.12 ± 0.07	213	0.17
G/A	0.14 ± 0.08	249	0.10 ± 0.06	292	0.30
G/G	0	351	0	587	0.51

[a]Deviation in Warner-Bratzler shear force.

the Cycle VII Angus sires indicated that the higher frequency of C/G haplotype was partially due to a sampling effect (59% in sires, 39% in bull panel), emphasizing the limited conclusions that should be drawn by frequencies within breed that depend on the small sample sizes available in the Cycle VII sires. With that in mind, however, the frequency in the Angus bull panel (39%) remained much larger than the average C/G haplotype frequency of the other sire breeds (7 ± 6%) or the frequency within any other single breed (range 0 to 19% within breed). This supported the conclusion that the C/G haplotype is more common in the Angus breed.

Association of SNP Markers with Shear Force Values

Analysis of both the 316 and 530 markers with WBSF values in the ASA sample indicated an association of genotype and phenotype for both markers (Table 1). Animals homozygous for the C allele at marker 316 had lower shear force ($P = 0.02$) than animals of CG or GG genotype, and animals with homozygous G genotype at marker 530 had lower shear force ($P = 0.04$) than animals of AG or AA genotype. The difference between mean shear force values of alternate homozygote classes was greater for the 316 marker (0.6 kg for the 316 marker vs. 0.4 kg for the 530 marker). A similar association of individual marker genotype and shear force was detected in the Cycle VII population. There was an effect of both the 316 and 530 markers on shear force ($P = 0.02$ and 0.01, respectively), with the difference between mean shear force values greater for the 316 marker (0.3 kg for the 316 marker vs. 0.2 kg for the 530 marker). Although the differences between the mean shear forces of alternate homozygous classes in the Cycle VII population were smaller than those observed in the ASA animals, the same marker alleles were associated with lower shear force. Moreover, these results are consistent with the original report on single-sire resource populations, in which the C/G haplotype of the sires was associated with lower shear force (Page et al., 2002).

The two groups of animals were also analyzed with both marker genotypes fitted simultaneously to determine whether the combination of markers improved reliability of the test. When a simultaneous model was analyzed for the ASA sample, animals homozygous CC for marker 316 and homozygous GG for marker 530 (i.e., homozygous for the C/G haplotype) had decreased

Table 4. Allele and haplotype frequencies of sires by breed (for Cycle VII sires) or American Simmental Association (ASA) and Angus bull panel sample

	Cycle VII sire breed frequency							Sample frequency	
Item panel	Angus	Charolais	Gelbvieh	Hereford	Limousin	Red Angus	Simmental	ASA	Angus bull
316 marker allele									
C	26	2	0	2	3	7	4	10	150
	(59%)	(5%)	(0%)	(6%)	(8%)	(19%)	(11%)	(14%)	(39%)
G	18	40	38	34	35	29	32	62	232
530 marker allele									
A	3	19	24	4	18	8	21	35	61
	(7%)	(45%)	(63%)	(11%)	(47%)	(22%)	(58%)	(49%)	(16%)
G	39	23	14	32	20	28	15	37	321
Haplotype									
C/A	0	0	0	0	1	0	1	2	0
	(0%)	(0%)	(0%)	(0%)	(3%)	(0%)	(3%)	(3%)	(0%)
C/G	24	2	0	2	2	7	3	33	150
	(57%)	(5%)	(0%)	(6%)	(5%)	(19%)	(8%)	(46%)	(39%)
G/A	3	19	24	4	17	8	20	8	61
	(7%)	(45%)	(63%)	(11%)	(45%)	(22%)	(56%)	(11%)	(16%)
G/G	15	21	14	30	18	21	12	29	171
	(36%)	(50%)	(37%)	(83%)	(47%)	(58%)	(33%)	(41%)	(45%)

shear force relative to that of animals homozygous GG for both markers (Table 2), and averaged a full kilogram lower shear force than the average for the class homozygous GG and AA at markers 316 and 530, respectively (i.e., homozygous for the G/A haplotype; $P < 0.03$). However, the low numbers of animals in some cells limits the confidence in the estimates of genotypic effects. For example, there were only six animals in the homozygous C/G haplotype class and no animals homozygous for C/A haplotype. The use of both markers simultaneously in Cycle VII identified three significant contrasts. Average shear force of animals containing homozygous G at marker 316 and heterozygous AG at marker 530 (a mixture of G/A and G/G haplotypes) was higher (0.2 kg) than for animals homozygous GG for both markers ($P = 0.02$), and higher (0.4 kg) than for animals homozygous CC and GG at markers 316 and 530, respectively (i.e., homozygous C/G haplotype; $P = 0.02$). The average shear force difference between animals homozygous for the C/G haplotype (CC marker 316, GG marker 530) and the G/A haplotype (GG marker 316, AA marker 530) also differed by 0.4 kg ($P = 0.06$). The higher average shear force for animals with G/A haplotype is consistent with previous results indicating that this haplotype was associated with increased shear force (Page et al., 2002). No other contrasts reached significance ($P > 0.10$).

A 3-df test contrasting two-marker haplotypes was applied to more directly examine whether haplotypes had significant effects on shear force (Table 3). Animals heterozygous at both markers were assigned the highest probability haplotypes based on overall haplotype frequencies in the sample for this analysis. The mean shear force for the C/G haplotype was approximately 0.3 kg ($P = 0.01$) and 0.2 kg ($P = 0.04$) lower than for the G/A haplotype in the ASA and Cycle VII animals, respectively.

Discussion

The creation of resource populations for QTL detection commonly applies the approach of using one or two crossbred sires to generate large, half-sib families. The effect of inheriting alternative sire alleles on production traits can then be ascertained. The family structure and size increases the power to detect QTL of moderate effect using a relative few genetic markers spread across the genome, and the use of a crossbred sire(s) increases the number of allele contrasts in the offspring. However, single-sire resource populations have more limited utility for evaluating specific SNP markers for potential use in selection because alleles of the sire for any marker in the chromosomal region carrying the QTL might be expected to show some association with phenotype. We previously made use of the dam alleles of the resource population to provide initial support for utility of the *CAPN1* markers (Page et al., 2002). The results reported herein expand on the previous work by examining the predictive merit of two *CAPN1* markers in populations that incorporate a wider variety of commercially relevant germplasm.

The first goal was to determine whether minor allele frequencies of the two SNP were sufficiently high in the ASA and Cycle VII populations to make it feasible to analyze the effect of alternative alleles. Determination that minor allele frequencies were ≥17% in both populations provides support for the analysis and suggests that there is sufficient room to change allele frequency in beef cattle to make a test at this locus worthwhile. Furthermore, the results indicate that variation at *CAPN1* is segregating in all seven of the most popular *Bos taurus* beef breeds in the United States.

Two-marker genotypes permitted evaluation of haplotype frequency among calves of the populations. Although no animals were homozygous for the C/A haplotype, the data in Table 2 demonstrate that all four possible haplotypes were observed at least once in this sample of animals, indicating that historical recombination between markers is likely to have occurred. The most recent draft of the human genome (Build 34 version 3, www.ncbi.nlm.nih.gov; accession NT_033903.6, Feb. 10, 2004), suggests that the two markers in *CAPN1* lie approximately 18.4 kb apart (because intron 10 of cattle has not been completely sequenced, distance in cattle must be inferred from human data), close enough to be in significant linkage disequilibrium, but far enough apart that it is reasonable to propose that recombination could have occurred at some time in evolution of cattle.

Analysis of the effect of the two *CAPN1* markers on shear force suggests that they have predictive merit when applied independently in the ASA sample. Furthermore, the simultaneous analysis of two-marker genotype effects suggests that animals homozygous for the C/G haplotype are associated with the most favorable shear force phenotype. The results in the Cycle VII population support this conclusion, although the magnitudes of the contrast in WBSF values are smaller than in the ASA sample, probably because of the multibreed nature of the population, or the different genetic backgrounds, or environmental effects. In the previous study of single-sire resource populations (Page et al., 2002), the magnitude of effect seemed much larger in the Jersey × Limousin population than was observed in the Piedmontese × Angus population, which was interpreted to be due at least in part to the larger overall variation in shear force values in the Jersey × Limousin population (Page et al., 2002). When used independently in this study, it seems that the 316 marker may have better predictive power than the 530 in the ASA population, which is the opposite conclusion relative to the previous study (Page et al., 2002), whereas the 530 marker had the more significant association in the Cycle VII population.

The predicted haplotype frequencies of Cycle VII sires showed differences depending on breed background, with the C/G haplotype much more common (59%) in the purebred Angus sires of Cycle VII compared with

the other breeds. The increased frequency seems to be a real breed effect as a similarly high percentage (39%) of C/G haplotype was observed in an independent panel of Angus animals. Bias in allele frequency among breeds in a multibreed research population raises the possibility of population stratification artifacts when performing marker association analyses such as those described in this study. Specifically, if Angus genetics contribute disproportionately to one extreme or the other of phenotype, the biased allele frequency could lead to a false association. It is important to note that differences in longissimus tenderness among the Cycle VII sire breeds were generally small (Wheeler et al., 2004). At constant age, LM from Angus-sired steers had lower 14-d postmortem WBSF values than did LM from steers of Gelbvieh or Charolais sired breeds, whereas sire breed did not affect trained sensory panel tenderness rating. Longissimus steaks of steers from Angus dams had slightly lower shear force compared with LM of steers from Hereford or MARC III dams (Wheeler et al., 2004). Some comparisons of sire breeds that have included Angus have found small but significant differences in LM tenderness between Angus and other breeds (Koch et al., 1979, 1982; Wheeler et al., 2001, 2004), whereas other comparisons have not detected differences between Angus and other sire breeds (Koch et al., 1976; Wheeler et al., 1996). Results from purebred steers have shown that LM from Angus was tenderer than that from Limousin, Gelbvieh, Simmental, and Charolais (Gregory et al., 1994). These findings are generally consistent with the higher frequency of the tenderer C/G haplotype in the Angus breed. This is why it was important that the statistical model properly account for breed background, which is in the model terms for sire breed, dam breed, and sire breed × dam breed interaction. The model was chosen to make the comparison between genotypes within breed classes, decreasing the effect of allele frequency bias and population stratification on the conclusions. Furthermore, the large ASA sample showed similar effects as the Cycle VII population, indicating that the association of *CAPN1* markers with phenotype is valid, and is unlikely to be the result of population stratification.

The previous study, which included Piedmontese × Angus and Jersey × Limousin populations (Page et al., 2002), contrasted the G/A haplotype (the sire allele originating from Piedmontese and Limousin lines, respectively) with the C/G haplotype (the sire allele originating from the Angus and Jersey lines, respectively). In both populations, inheritance of the G/A allele from the sire was found to be associated with increased shear force relative to the C/G allele. The G/G haplotype allele was not directly examined in the previous study (Page et al., 2002,) as neither of the sires used carried this allele, although it is the predominant haplotype in all of the breeds making up Cycle VII, except for Angus (the 21 purebred Angus sires had 57% C/G haplotype alleles and 36% G/G haplotype alleles). Our results support the conclusion that the C/G haplotype is associated with a favorable shear force phenotype, and suggest that the G/G haplotype may have intermediate effects between the C/G and G/A haplotypes or may be a mixture of functionally distinct alleles. The scarcity of C/A haplotype alleles does not permit rigorous evaluation of its relative effect on shear force, although the data on the ASA population, analyzed with two-marker genotypes fit simultaneously, suggest that the effect may be favorable relative to the G/G or G/A haplotype.

The results reported here extend the previous study by demonstrating the predictive merit of the 316 and 530 *CAPN1* markers outside of the original resource populations. In addition, the results suggest that selection for C/G haplotype would have a favorable effect on shear force measurements in commercial cattle. Frequency data from both populations suggest that this favorable allele is present in all seven breeds at estimated frequencies ranging from approximately 10 to 40%, indicating that selection could have an effect on favorable allele frequency in major beef cattle breeds. However, it is important to note that neither population in this study represents *Bos indicus*-influenced breeds. A resource population established with a Brahman × Hereford crossbred bull has demonstrated the presence of a QTL on bovine chromosome 29 in the area of the *CAPN1* gene (Casas et al., 2003), but sequencing of the gene has revealed that the bull was homozygous for both 316 and 530 markers (E. Casas and B. Page, unpublished data). In addition, preliminary work with a Santa Gertrudis cross population has suggested that the markers may not perform as expected from the data reported in this study (R. L. Quaas, unpublished data). Further work characterizing the *CAPN1* haplotypes in *Bos indicus*-influenced cattle is underway to determine appropriate molecular marker systems to track functional alleles.

Implications

Genetic markers for the *CAPN1* gene have been previously published and associated with meat tenderness, but in relatively artificial research populations with a narrow genetic base. Our study demonstrates the predictive merit of these markers in commercial beef cattle and indicates that the favorable alleles are present in all the most popular *Bos taurus* beef breeds but at low to intermediate frequency. These results also provide the basis for the most efficient selection by indicating the two-marker haplotype likely to be most consistently associated with a favorable functional allele. This provides the opportunity to use genetic markers to improve longissimus muscle tenderness. However, additional work is required to evaluate these markers in other breeds or to identify other more appropriate markers for those breeds; this is especially true for *Bos indicus*-influenced cattle.

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