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# Validation of commercial DNA tests for quantitative beef quality traits<sup>1,2</sup>

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**ABSTRACT:** Associations between 3 commercially available genetic marker panels (GeneSTAR Quality Grade, GeneSTAR Tenderness, and Igenity *TenderGENE*) and quantitative beef traits were validated by the US National Beef Cattle Evaluation Consortium. Validation was interpreted to be the independent confirmation of the associations between genetic tests and phenotypes, as claimed by the commercial genotyping companies. Validation of the quality grade test (GeneSTAR Quality Grade) was carried out on 400 Charolais × Angus crossbred cattle, and validation of the tenderness tests (GeneSTAR Tenderness and Igenity *TenderGENE*) was carried out on over 1,000 *Bos taurus* and *Bos indicus* cattle. The GeneSTAR Quality Grade marker panel is composed of 2 markers (TG5, a SNP upstream from the start of the first exon of thyroglobulin, and QG2, an anonymous SNP) and is being marketed as a test associated with marbling and quality grade. In this validation study, the genotype results from this test were not associated with marbling score; however, the association of substituting favorable alleles of the marker panel with increased quality grade

(percentage of cattle grading Choice or Prime) approached significance ( $P \leq 0.06$ ), mainly due to the effect of 1 of the 2 markers. The GeneSTAR Tenderness and Igenity *TenderGENE* marker panels are being marketed as tests associated with meat tenderness, as assessed by Warner-Bratzler shear force. These marker panels share 2 common  $\mu$ -calpain SNP, but each has a different calpastatin SNP. In both panels, there were highly significant ( $P < 0.001$ ) associations of the calpastatin marker and the  $\mu$ -calpain haplotype with tenderness. The genotypic effects of the 2 tenderness panels were similar to each other, with a 1 kg difference in Warner-Bratzler shear force being observed between the most and least tender genotypes. Unbiased and independent validation studies are important to help build confidence in marker technology and also as a potential source of data required to enable the integration of marker data into genetic evaluations. As DNA tests associated with more beef production traits enter the marketplace, it will become increasingly important, and likely more difficult, to find independent populations with suitable phenotypes for validation studies.

**Key words:** beef quality, commercial DNA test, genetic marker, validation

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## INTRODUCTION

Gene mapping and discovery programs have resulted in the detection of a plethora of QTL for various beef cattle production traits. Because of the cost of collecting phenotypes and genotypes from large animals, discov-

ery populations often involve a relatively small sample size (400 to 500 animals). Before moving genetic markers from discovery populations to commercialization, it is important to validate their purported effects on the

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<sup>2</sup>Reference herein to any specific commercial products by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the US government or the NBCEC. The views and opinions of authors expressed herein do not necessarily state or reflect those of the US government or the NBCEC, and shall not be used for advertising or product endorsement purposes.

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**Table 1.** Phenotypic values for marbling score and Warner-Bratzler shear force collected from >7,000 progeny sired by bulls of 14 breeds as a part of the Carcass Merit Project

Trait	Mean	Minimum	Maximum	SD
Marbling <sup>1</sup>	420	190	900	97.09
Shear force, kg	4.61	1.84	13.14	1.51

<sup>1</sup>400 = Small<sup>00</sup>, 500 = Modest<sup>00</sup>.

trait of interest in different breeds and environments, and assess them for correlated responses in the associated traits (Barendse, 2005). One of the biggest challenges in achieving this objective is the paucity of cattle populations with sufficient phenotypic data to assess the association between various traits and newly discovered genetic markers, and this makes it difficult and expensive to do large-scale field evaluations.

Results from such validation studies to date have not been widely published (Burrow and Bindon, 2005), and genetic marker tests may sometimes be commercialized before the collection of any field validation data. This fact, in conjunction with conflicting reports about some commercially available markers (Barendse et al., 2005; Casas et al., 2005c) and the recognized occurrence of well-proven bulls with a high EPD for a given trait but carrying 2 copies of the wrong (unfavorable) marker allele for that trait, have made some producers understandably wary of investing in DNA-based testing. Producers want to know whether DNA-based tests perform according to the claims of the marketing company and are interested in third-party, independent validation of these tests.

The results of validation studies with 3 commercially available genetic tests (GeneSTAR Quality Grade, GeneSTAR Tenderness, and Igenity *TenderGENE*) are reported in this study.

## MATERIALS AND METHODS

### Validation Process

The National Beef Cattle Evaluation Consortium (NBCEC, <http://www.nbcec.org>, last accessed 22 December 2006) has been charged with undertaking a validation process of commercially available genetic tests for beef cattle production traits in the United States. The NBCEC has defined validation to mean the independent verification of associations between genetic tests and phenotypes, as claimed by the commercial genotyping company. This is done through the analysis of phenotypes and genotypes derived from reference cattle populations. The genotyping company requests validation of their claims and is responsible for genotyping the DNA samples. The NBCEC then performs an analysis to determine whether there is an association between the results of the genetic test and the phenotype for the claimed trait. The validation pro-

cess is, therefore, a collaboration of the owners of the DNA and the phenotypes (e.g., breed associations) and the commercial testing companies, facilitated by the NBCEC.

### DNA Testing Companies and Sample Populations

Phenotypic data and DNA were mostly derived from samples collected for the Carcass Merit Project (CMP), as previously described (Minick et al., 2004). Animal Care and Use Committee approval was not obtained for this study because the DNA and data were obtained from these preexisting CMP samples (CMP DNA repository at Texas A&M University). All animals were harvested under 30 mo of age. Some contemporary groups consisted of steers and others of heifers. These are field data representing a cross-section of commercial cattle sired by the denoted breeds raised under different management practices and environmental conditions. Such diversity of breeds and environments is likely to be typical of commercial applications of DNA testing. These data are owned by the various participating breed associations. Each commercial testing company selected the breed groups to be used for the validation and then reached an agreement with the respective breed associations. Ideally, the analyses included *Bos taurus* and *Bos indicus* reference populations, although such populations with the appropriate phenotypes and allele frequencies were not readily available.

Bovigen LLC (Harahan, LA) chose to validate their 2 GeneSTAR marker panels on Charolais- and Hereford-sired CMP cattle. The former (n = 400) were from commercial Angus dams; and the latter (n = 285) were primarily from Hereford or Hereford × Red Angus dams. Additionally the GeneSTAR Tenderness panel was validated on 2 populations of Brahman-sired cattle (Brahman dams, n = 674). Approximately half of the Brahmans (n = 318) were CMP cattle from the USDA-ARS SubTropical Agricultural Research Station (STARS) in Brooksville, FL. The remaining Brahman cattle were the offspring of 68 Brahman bulls bred to Brahman cows, and the data were collected as a part of a research project at the Louisiana State University Agricultural Center (Smith et al., 2005; Beauchemin et al., 2006).

Merial (Duluth, GA) used the same CMP Charolais-sired and Brahman-sired cattle populations, plus CMP cattle sired by Red Angus (Red Angus and Red Angus-cross dams; n = 310) and Brangus (Brangus and Brangus-cross dams; n = 181) bulls for their Igenity *TenderGENE* test validation.

### DNA Tests

The GeneSTAR Quality Grade marker panel is composed of a C/T SNP in the consensus binding sequence for RNA polymerase III, 537 bp upstream from the start of the first exon of thyroglobulin (TG5, position 422 of accession # X05380; Barendse et al., 2004) and an anonymous SNP (QG2, unpublished data). The Gene-

STAR Tenderness panel is composed of a G/A SNP in the 3' untranslated region of calpastatin (**CAST-T1**, base 2959 of accession # AF159246; Barendse, 2002), a G/C SNP in exon 9 of  $\mu$ -calpain (**CAPN1 316-T2**, base 5709 of accession # AF252504; Page et al., 2002) that produces an amino acid substitution (the C allele codes for alanine, the G allele codes for glycine), and a C/T SNP in the intron between the 17th and 18th exon of  $\mu$ -calpain (**CAPN1 4751-T3**, base 6545 of accession # AF248054; White et al., 2005). The Igenity *TenderGENE* marker panel consists of the 2  $\mu$ -calpain SNP described previously, and a calpastatin SNP (**UoG-CAST**; Schenkel et al., 2006). The UoG-CAST marker is a G/C SNP in the intronic sequence between exon 5 and 6 of calpastatin (base 282 of accession # AY008267). The 2 tenderness panels, therefore, share 2 common  $\mu$ -calpain SNP, and although each has a calpastatin SNP, these SNP reside at different loci. All genotyping was done by the respective companies.

### Phenotypes

The traits analyzed were the Warner-Bratzler shear force (**WBSF**) and the subjectively recorded marbling score (Minick et al., 2004; Dikeman et al., 2005) of the longissimus lumborum muscle. See Table 1 for the mean and dispersion statistics from the CMP cattle. American Meat Science Association guidelines (AMSA, 1995) were used for WBSF evaluation. Longissimus muscle sections were harvested at 24 to 48 h postmortem from numerous processing plants, with most using relatively high-voltage electrical stimulation. Subcutaneous fat, and bone and superficial muscles were removed, and steaks (2.54-cm thick) were vacuum-packaged and aged at 1 to 2°C until 14 d postmortem. The steaks were cooked to an internal temperature of 71°C in a Blodget forced-air, convection-gas oven (model DFG-201, G.S. Blodget Co. Inc., Burlington, VA) at 163°C. The internal temperatures were monitored by 30-ga., type-T copper and constantan wire, thermocouple probes connected to a Doric temperature recorder (Model 205, Vas Engineering, San Francisco, CA). The steaks were turned over once at an internal temperature of 35°C.

After reaching the end point temperature, the steaks were cooled at 1 to 2°C for 24 h, and six to eight 1.27-cm-diameter cores were removed parallel to the muscle fiber orientation and sheared with a WBSF V-blade attached to an Instron Universal Testing Machine (model 4201, Instron Corporation, Canton MA) with a 50-kg compression load cell and a crosshead speed of 250 mm/min. The WBSF values for the 6 to 8 cores were averaged and used in the statistical analyses. A numeric score was used to record marbling, with 300 corresponding to Slight<sup>00</sup>, 400 corresponding to Small<sup>00</sup>, 500 corresponding to Modest<sup>00</sup>, etc. (AMSA, 1995). Quality grade was also analyzed as the percentage qualifying as USDA quality grades of Choice or Prime. This determination was based entirely on a marbling score

≥500 according to the US Standards for Grades of Carcass Beef (USDA, 1997) because all of the carcasses were chronologically of maturity A (i.e., <30 mo old).

### Statistical Analyses

The basic model was  $y = CG + \text{marker effect} + \text{sire} + e$ , where CG denotes a fixed contemporary group (same breed type, feedlot, sex, and slaughter date) and sire was a random effect. The parameters of interest were the marker effects; other effects in the model were nuisance parameters included to account for various factors possibly affecting the dependent variables. Within a contemporary group, the management of the cattle was the same.

The slaughter date was included to ensure that days on feed was constant and that at slaughter the cattle would have similar management conditions within a CG. The former was felt to be important for marbling-related traits, the latter for the tenderness trait. These data were not useful for detailed study of polygenic effects because of limited total numbers and numbers of sires per breed. A random sire effect was included to account for the expected covariances of paternal half-sibs.

The marker effect for the GeneSTAR Quality Grade panel was defined 2 ways. In the first definition, the effects were assumed equal and additive, so that the marker effect was the regression on the number of favorable alleles summed across markers. The second definition also assumed additivity but allowed for a different magnitude of marker effects (i.e., included a regression on the number of favorable alleles for each marker locus). For GeneSTAR Tenderness and Igenity *TenderGENE* marker panels, the marker effect was similar to the second definition above, except that, because there were 2 linked markers (i.e., *CAPN1* 316 & 4751), the regression was on the expected number of copies of each of the 4 haplotypes (1 of which was rare). Haplotype frequencies were estimated and analyses carried out with PROC HAPLOTYPE and PROC MIXED, respectively (SAS Inst. Inc., Cary, NC). The contrast procedure was used to jointly test the effect of the calpain haplotypes (3 df) and the calpain haplotype plus calpastatin (4 df).

Power was computed by the Kononoff and Hanford (2006) procedure, which requires specification of the variance components (sire and residual), the probability of type I error ( $\alpha$ ), and the size of an effect in actual units. Here  $\alpha = 0.05$ , variance component estimates were from the PROC MIXED analyses of the real data, and the allele substitution effect sizes were set to range between 0.05 and 0.50 of a phenotypic SD. The SD of 100 for Marbling Score and 1.5 kg for WBSF were taken from Table 1 (Dikeman et al., 2005).

## RESULTS

### Allele Frequencies

The sample genotype, haplotype, and allele frequencies and tests for Hardy-Weinberg equilibrium for each

of the SNP included in the commercial tests involved in this validation study are shown in Tables 2 and 3. The observed genotypic frequencies were in agreement with Hardy-Weinberg equilibrium. Some alleles and haplotypes were extremely rare (<0.5%) in certain populations (e.g., *CAPN1* 316/4751 C-T haplotype). Also included in the tables are allele and haplotype frequencies that have been reported in the literature for different cattle populations.

### GeneSTAR Quality Grade

One of the QG2 alleles was almost fixed in the Hereford-sired sample population, so the analysis included only the Charolais-sired  $\times$  Angus sample population (Table 2). The genotype results from the GeneSTAR Quality Grade test were not associated with marbling score; however, an increase in quality grade (percentage grading Choice or Prime) that approached significance ( $P \leq 0.06$ ) was associated with substituting the favorable allele of TG5 in the Charolais  $\times$  Angus crossbred animals (Table 4). The association of the test with quality grade was primarily attributable to the effect of the favorable allele of the TG5 marker. In this sample population, each TG5 star (favorable allele) was associated with an 8.6% increase in the number of cattle grading Choice or Prime, and each QG2 star was associated with a 2.9% increase in the number of cattle grading Choice or Prime. The average effect of a GeneSTAR Quality Grade star was associated with a 6.2% increase in the number of cattle grading Choice or Prime.

### GeneSTAR Tenderness

Improved tenderness was associated with substituting a T allele at CAST-T1 and a C allele at both  $\mu$ -calpain loci. The GeneSTAR Tenderness analysis included 1,302 animals (372 Charolais  $\times$  Angus, 260 Hereford, and 670 Brahman). The associations of CAST-T1 ( $P < 0.01$ ) and the  $\mu$ -calpain haplotype ( $P < 0.001$ ) with WBSF were each significant, as was the combination of these markers ( $P < 0.001$ ). Each calpastatin T was associated with a decrease of 0.15 kg in WBSF, and substituting the Calpain T2-T3 C-C haplotype for the Calpain T2-T3 G-T haplotype was associated with a decrease of 0.34 kg in WBSF (Table 5).

### Igenity TenderGENE

Improved tenderness was associated with substituting a C allele at UoG-CAST and a C allele at both  $\mu$ -calpain loci. The association of the UoG-CAST ( $P < 0.001$ ) and the  $\mu$ -calpain haplotype ( $P < 0.001$ ) with WBSF were each highly significant, and the combination of all 3 even more so ( $P < 0.001$ ). Table 6 shows the improvement in WBSF for each of the possible alleles or haplotypes contrasted to the least tender genotype (i.e., UoG-CAST GG, *CAPN1* 4751 TT, *CAPN1* 316 GG) calculated from a combined analysis of 1,209 cattle: 181 Brangus, 400 Charolais  $\times$  Angus crosses, 310 Red An-

gus, and 318 Brahman. In this sample population, each calpastatin C was associated with a decrease of 0.19 kg in WBSF, and substituting the *CAPN1* 4751 C/316 C haplotype for the *CAPN1* 4751 T/316 G haplotype was associated with a decrease of 0.33 kg in WBSF.

Combined genotypic effects for the GeneSTAR Tenderness and Igenity TenderGENE panels are presented in Table 7. There was a 1-kg difference in WBSF between the most and least tender genotypes in both panels.

## DISCUSSION

Genotypic and allele frequencies reported for each of the SNP examined in this study are summarized in Tables 2 and 3. These frequencies should be interpreted with care because they are often derived from research populations or crossbred cattle and are therefore not necessarily reflective of any purebred population. They do give some indication as to the general prevalence of the favorable marker in a range of breeds, and in the absence of data from purebred populations, these frequencies at least provide some indication as to genotypic frequencies in different types of cattle. This is important because the potential impact of selecting for a genetic marker depends on both the magnitude of its effect and its frequency in the population (Notter, 2004). All 3 of the genetic tests involved a marker panel (i.e., the test involved genotyping more than 1 marker locus), and the substitution effects of the markers or marker haplotypes in the panel were analyzed. For GeneSTAR Quality Grade, marker loci were analyzed assuming equal and additive effects because it is being marketed and used under the assumption that all loci are equal. Results where the effects of the loci were allowed to differ in their magnitude are also presented. This information is important to provide some indication as to the relative importance of each of the different loci included in the panel.

The favorable T allele of TG5 has been associated with increases in marbling score in both long-fed (250+ days on feed; Barendse, 1999), and short fed (<250 d on feed) Angus and Shorthorn animals where the genotype at this locus accounted for 6.5% of the residual variance for the marbling phenotype (Barendse et al., 2004). The frequency of the favorable TG5 allele is greatest in the Wagyu breed, intermediate in other *Bos taurus* breeds, and lowest in *Bos indicus* breeds (Table 2). The results of our study did not show a significant association of this marker with marbling score, but there was a trend toward increased quality grade associated with substituting the favorable allele of TG5 in Charolais  $\times$  Angus crossbred animals that had been fed for <250 d. The binary trait of % Choice and Prime represents a considerable loss of information compared with the continuous trait of marbling score. The association of quality grade with the results from this test in the absence of a significant association with marbling score was probably the result of a high proportion of

**Table 2.** Genotypic (for the favorable allele) and allelic frequencies for SNP marker loci and tests for Hardy-Weinberg equilibrium

Marker	Favorable allele	Population description	Genotype, %			No. of cattle	Frequency		Hardy-Weinberg <i>P</i> -value	Reference
			0	1	2		Unfavorable allele	Favorable allele		
CAST-T1	T	Charolais × Angus	1	11	88	409	0.06	0.94	0.25	Present study
		Hereford	16	50	34	322	0.41	0.59	0.88	Present study
		Brahman	11	46	43	674	0.34	0.66	0.84	Present study
		Angus	1	21	78	1,078	0.12	0.89	0.70	GeneNote 4
		Hereford	2	28	70	733	0.16	0.84	0.64	GeneNote 4
		Shorthorn	0.5	2.5	97	298	0.02	0.98	0.27	GeneNote 4
		Murray Gray	1	18	81	357	0.10	0.90	1.0	GeneNote 4
		Brahman	18	50	32	768	0.43	0.57	0.88	GeneNote 4
		Belmont Red	4	35	61	1,137	0.22	0.79	0.47	GeneNote 4
		Santa Gertrudis	8	37	55	1,014	0.27	0.74	0.31	GeneNote 4
		Cycle VII <sup>1</sup>	4	31	65	539	0.20	0.80	0.97	Casas et al., 2006
		Cycle VIII <sup>2</sup>	2	29	69	580	0.17	0.83	0.57	Casas et al., 2006
		STARS (Brahman) <sup>3</sup>	6	44	50	444	0.28	0.72	0.21	Casas et al., 2006
		Sanga	8	24	68	25	0.20	0.80	0.76	Burrow, 2003
		Sanga-derived	3	25	73	40	0.15	0.85	0.90	Burrow, 2003
UoG-CAST	C	Charolais × Angus	5	33	62	412	0.21	0.79	0.92	Present study
		Brangus	5	32	63	203	0.21	0.79	0.96	Present study
		Red Angus	8	36	56	305	0.26	0.74	0.64	Present study
		Brahman	33	47	20	344	0.57	0.43	0.76	Present study
		Angus <sup>4</sup>	—	—	—	12	0.38	0.63	—	Schenkel et al., 2006
		Limousin	—	—	—	28	0.27	0.73	—	Schenkel et al., 2006
		Charolais	—	—	—	8	0.31	0.69	—	Schenkel et al., 2006
		Simmental	—	—	—	33	0.64	0.36	—	Schenkel et al., 2006
		Other <sup>5</sup>	—	—	—	547	0.36	0.63	—	Schenkel et al., 2006
		Combined	17	39	43	628	0.37	0.63	0.00	Schenkel et al., 2006
CAPN1 4751	C	Charolais × Angus	27	54	19	435	0.54	0.46	0.22	Present study
		Brangus	20	51	29	219	0.45	0.55	0.95	Present study
		Red Angus	26	54	21	307	0.53	0.47	0.46	Present study
		Hereford	71	25	4	305	0.84	0.16	0.37	Present study
		Brahman	88	11	1	674	0.94	0.06	0.07	Present study
		Cycle VII	19	47	34	550	0.42	0.58	0.70	White et al., 2005
		Cycle VIII	12	48	40	596	0.36	0.64	0.65	White et al., 2005
CAPN1 316	C	STARS (Brahman)	81	19	0	471	0.90	0.10	0.13	White et al., 2005
		Charolais × Angus	58	37	4	435	0.77	0.23	0.43	Present study
		Brangus	67	31	2	217	0.82	0.18	0.61	Present study
		Red Angus	59	36	5	307	0.77	0.23	0.97	Present study
		Brahman	96	4	0	674	0.98	0.02	0.90	Present study
		Hereford	56	40	4	309	0.76	0.24	0.25	Present study
		Angus	—	—	—	213	0.59	0.41	—	Page et al., 2004
		Hereford	—	—	—	17	0.94	0.06	—	Page et al., 2004
		Red Angus	—	—	—	18	0.81	0.19	—	Page et al., 2004
		Limousin	—	—	—	19	0.92	0.08	—	Page et al., 2004
		Simmental	—	—	—	54	0.87	0.13	—	Page et al., 2004
		Charolais	—	—	—	21	0.95	0.05	—	Page et al., 2004
		Gelbvieh	—	—	—	19	1.00	0.00	—	Page et al., 2004
		Cycle VII	64	32	5	532	0.80	0.20	0.99	White et al., 2005
		Cycle VIII	58	37	5	599	0.76	0.24	0.81	White et al., 2005
TG5	T	STARS (Brahman)	97	3	0	470	0.98	0.02	0.48	Casas et al., 2005
		Charolais × Angus	62	34	5	409	0.78	0.22	0.98	Present study
		Hereford	81	18	1	324	0.90	0.10	0.99	Present study
		STARS (Brahman)	95	4	1	467	0.97	0.03	0.00	Casas et al., 2005
		Angus	64	32	3	819	0.81	0.19	0.56	Barendse et al., 2004
		Shorthorn	67	31	3	744	0.82	0.18	0.77	Barendse et al., 2004
		Mixed breed <sup>6</sup>	61	34	5	99	0.78	0.22	0.98	Barendse et al., 2004
		Angus	50	39	11	—	0.70	0.31	—	GeneNote 1
		Wagyu	12	50	38	—	0.37	0.63	—	GeneNote 1
		Other breeds <sup>7</sup>	62	30	8	—	0.77	0.23	—	GeneNote 1
		Black Angus	53	38	9	>100	0.72	0.28	—	Nicol et al., 2001
		Red Angus	42	42	16	>100	0.63	0.37	—	Nicol et al., 2001
		Wagyu	12	50	38	>100	0.37	0.63	—	Nicol et al., 2001
		Angus-based M1 line <sup>8</sup>	63	31	7	134	0.78	0.22	0.63	Moore et al., 2003

*Continued*

**Table 2 (Continued).** Genotypic (for the favorable allele) and allelic frequencies for SNP marker loci and tests for Hardy-Weinberg equilibrium

Marker	Favorable allele	Population description	Genotype, %			No. of cattle	Frequency		Hardy-Weinberg <i>P</i> -value	Reference
			0	1	2		Unfavorable allele	Favorable allele		
M2	?	Holstein	61	29	11	28	0.75	0.25	0.69	Thaller et al., 2003
		Charolais	56	41	4	27	0.76	0.24	0.98	Thaller et al., 2003
		Sanga	100	0	0	25	1.00	0.00	1.00	Burrow, 2003
		Sanga-derived	90	10	0	39	0.95	0.05	0.43	Burrow, 2003
		Charolais × Angus	63	33	4	420	0.79	0.21	0.99	Present study
		Hereford	97	3	0	311	0.99	0.01	0.27	Present study

<sup>1</sup>Cycle VII, 564 crossbred steers of *Bos taurus* descent generated by AI with semen of bulls from the 7 beef breeds with the greatest number of registered animals in the United States (Hereford, Angus, Red Angus, Simmental, Gelbvieh, Limousin, Charolais; Page et al., 2004b).

<sup>2</sup>Cycle VIII, 580 crossbred steers 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 MARC III cows (Casas et al., 2006a).

<sup>3</sup>STARS (Brahman) population of 504 Brahman calves managed by the SubTropical Agricultural research station (Riley et al., 2002).

<sup>4</sup>Breed in this study was defined as >5/8 for a given breed.

<sup>5</sup>Other animals with breed composition <5/8 for all breeds (breed composition included Angus, Limousin, Charolais, and Simmental).

<sup>6</sup>Mixed breed = Murray Gray, Red Angus, crossbred Angus, Shorthorn, Charolais, Shaver, Limousin, Simmental, Santa Gertrudis, and Red Composite.

<sup>7</sup>Other breeds = not stated.

<sup>8</sup>Angus-based M1 line of Beefbooster Inc. (Calgary, Canada).

animals on the border of the USDA Select/Choice cutoff. The absolute improvement in quality grade associated with any marker will always be dependent upon marbling end point. This emphasizes the importance of environmental and management variables on the results derived from validation studies.

Three other peer-reviewed studies have examined associations of beef quality traits with the TG5 polymorphism. There was no association found between this marker and backfat in *Bos taurus* cattle (Moore et al., 2003), marbling score in *Bos indicus* cattle (Casas et al., 2005), or intramuscular fat in the longissimus muscle in a very small sample ( $n = 27$ ) of German Charolais (Thaller et al., 2003). However, an association was found between TG5 and both fat thickness and longissimus area in *Bos indicus* cattle (Casas et al., 2005), and longissimus intramuscular fat (but not semitendinosus muscle) in a small sample ( $n = 28$ ) of German Holstein cattle (Thaller et al., 2003). The second locus in the GeneSTAR Quality Grade panel is known simply as anonymous marker QG2. Although it is understandable that companies would want to protect their intellectual property from unauthorized use, such anonymity makes it difficult to compile some of the information

that is important in using the marker in breeding programs (e.g., the frequency of the favorable allele in a range of breeds). In our study with Charolais × Angus crossbred cattle, the association between the GeneSTAR Quality Grade test and quality grade was primarily attributable to the effect of the favorable allele of the TG5 marker.

Calpastatin and  $\mu$ -calpain are enzymes involved in the calpain proteolytic enzyme system responsible for postmortem meat tenderization (Koochmaraie, 1996). A recent study reported that CAST-T1 was associated with WBSF and tenderness score in *Bos taurus* and *Bos taurus* × *Bos indicus* crossbred cattle; however, there was no significant association in a purebred *Bos indicus* population (Casas et al., 2006). The *CAPN1* 316 and 4751 haplotype was also highly significantly associated with tenderness in our study. The association of *CAPN1* 316-T2 with meat tenderness in *Bos taurus* and *Bos taurus* × *Bos indicus* crossbred cattle has been found in other studies (Page et al., 2002, 2004). The *CAPN1* 4751 has been found to be a useful marker in cattle of all subspecies backgrounds, and haplotype analyses have consistently found that the lowest WBSF is associated with the *CAPN1* 316/4751 C-C haplotype

**Table 3.**  $\mu$ -Calpain 316/4571 haplotype frequencies

Population description	$\mu$ -Calpain 316/4571 haplotype				No. of cattle	Reference
	G-T	C-T	G-C	C-C		
Charolais × Angus	0.51	0.03	0.26	0.20	400	Present study
Brangus	0.45	0	0.37	0.17	181	Present study
Red Angus	0.51	0.01	0.25	0.23	310	Present study
Brahman	0.92	0	0.07	0.02	318	Present study
Hereford	0.12	0.04	0.64	0.20	260	Present study
Cycle VII	0.41	0.01	0.38	0.20	526	White et al., 2005
Cycle VIII	0.35	0.01	0.42	0.22	595	White et al., 2005

**Table 4.** Association between GeneSTAR Quality Grade panel results and marbling score and quality grade (% of animals grading Choice and Prime) phenotypes from 387 Charolais-sired × Angus cattle

Trait	Marker	Frequency, favorable allele	Estimate, effect	SE	P-value
Marbling score	GeneSTAR Quality Grade <sup>1</sup>		5.7	4.2	0.18
	TG5 <sup>2</sup>	0.22	9.7	5.9	0.10
	QG2 <sup>2</sup>	0.21	0.1	7.0	0.99
Choice and prime, %	GeneSTAR Quality Grade <sup>1</sup>		6.2	3.2	0.06
	TG5 <sup>2</sup>	0.22	8.6	4.5	0.06
	QG2 <sup>2</sup>	0.21	2.9	5.2	0.58

<sup>1</sup>Combined 2-marker panel = total number of favorable TG5 and QG2 alleles; value of an average favorable allele.

<sup>2</sup>Individual effects of TG5 and QG2 favorable alleles.

(White et al., 2005). The *CAPN1* 316/4751 C-T haplotype is extremely rare in *Bos taurus* and *Bos indicus* populations (Table 3). The effect of the *CAPN1* haplotype on WBSF was greater than the effect of the CAST-T1 marker.

The average allele substitution effect of a G to C substitution at UoG-CAST was  $-0.19$  kg in WBSF in our study, in agreement with Schenkel et al. (2006) who found a range from  $-0.26$  to  $-0.13$  kg for WBSF evaluated at 2 and 21 d of aging, respectively. The genotypic effects of the 2 tenderness panels, GeneSTAR Tenderness and Igenity *TenderGENE*, were very similar to each other (Table 7), suggesting that the 2 calpastatin SNP are marking the same tenderness-associated region of the genome. The magnitude of the WBSF reduction is distinctly greater than the difference in tenderness that has been recorded between Select and low Choice quality grades and is even greater than the tenderness difference between Select and premium Choice (upper two-thirds of Choice; Smith et al., 1985; Shackelford et al., 1994; NLSMB, 1995). From the perspective of genetic improvement, it is interesting to observe that the frequency of the *CAPN1* 316/4751 G-T haplotype is relatively high (Table 2). This suggests that the beef industry may have the opportunity to make improvement in tenderness by increasing the frequency of the *CAPN1* 316/4751 C-C haplotype.

Power results are presented in Table 8. The relatively small sample for GeneSTAR Quality Grade marker panel had the power to detect a marbling score substitution effect greater than about 20 units (0.20 SD). The larger data sets for WBSF were more powerful, allowing efficient detection of effects of about 0.15 to 0.23 kg (0.10 to 0.15 SD). Comparing the tenderness markers, there was less power to detect a *CAPN1* 316 effect even though the sample sizes were the same. This illustrates that for detecting marker effects, allele frequencies are perhaps as critical as sample size. Unfortunately, there are often no good estimates of allele frequencies for different breeds. For example, in this study, the Hereford sample was genotyped for the GeneSTAR Quality Grade panel, but these data were not included in the validation because of the almost total lack of polymorphism at the QG2 locus.

No single study can examine all of the breeds, allele frequencies, or environmental and management conditions that may factor into whether an association is found between a marker and trait. Validation studies are therefore problematic because findings are dependent on the specific characteristics of the finite number of populations screened. Power is only one of the variety of reasons why a DNA test might fail to validate (i.e., no association is found between the marker and the trait of interest). Other potential reasons for inconsis-

**Table 5.** Association between GeneSTAR Tenderness panel results and tenderness (Warner-Bratzler shear force, kg) phenotypes from 372 Charolais-sired × Angus, 260 Hereford, and 670 Brahman cattle

No. of cattle	Marker	Allele/haplotype	Sample frequency	Estimated effect, kg	SE
1,302	CAST-T1	T	0.72	-0.15	0.11
		C	0.28	0.00	
1,302	CAPN1 T2-T3 (316-4751)	C-C	0.11	-0.34	0.17
		C-T <sup>1</sup>	0.02	-0.16	0.38
		G-C	0.23	-0.18	0.14
		G-T	0.64	0.00	

<sup>1</sup>The low number of animals with the C-T haplotype in this study made it difficult to derive an accurate estimate of its effect.



**Table 6.** Association between Igenity *TenderGENE* panel results and tenderness (Warner-Bratzler shear force, kg) phenotypes from 181 Brangus, 400 Charolais-sired × Angus-cross, 310 Red Angus, and 318 Brahman cattle

No. of cattle	Marker	Allele/haplotype	Sample frequency	Estimated effect, kg	SE
1,209	UoG-CAST	C	0.72	-0.19	0.05
		G	0.28	0.00	0.00
1,209	CAPN1 (316-4751)	C-C	0.16	-0.33	0.07
		C-T <sup>1</sup>	0.01	0.22	0.23
		G-C	0.22	-0.18	0.06
		G-T	0.61	0.00	

<sup>1</sup>The low number of animals with the C-T haplotype in this study made it difficult to derive an accurate estimate of its effect.

tent results include differing marker-trait linkage phases, genotype × environment interactions, and epistatic effects (Dekkers, 2004). Obviously, there may be no real association between the DNA test and the traits upon which effects were claimed. Another possibility is that the discovery population may not have included breed composition or pedigree in the analysis, perhaps

because they were not known, as may often be the case in field data. This can lead to spurious associations that are due to population stratification. For example, if the population is a mixture of 2 breeds that differ for a trait of interest, and also in allele frequencies at a putative DNA test, but not due to linkage disequilibrium, a spurious association in the combined data (ignoring breed)

**Table 7.** Combined 3-marker genotypic effects on Warner-Bratzler shear force (kg), SE, and frequencies for the GeneSTAR Tenderness and Igenity *TenderGENE* panels as estimated from 1,302 (372 Charolais-sired × Angus, 260 Hereford, and 670 Brahman), and 1,209 (181 Brangus, 400 Charolais-sired × Angus-cross, 310 Red Angus, and 318 Brahman) cattle, respectively

GeneSTAR's T1 or Igenity's UoG-CAST1	Genotype		GeneSTAR Tenderness				Igenity <i>TenderGENE</i>			
	T2 =	T3 =	Estimate,				Estimate,			
	CAPN1 316	CAPN1 4751	kg	SE	No.	%	kg	SE	No.	%
2 or CC	2 = CC	2 = CC	-1.0	0.2	11	0.8	-1.0	0.2	18	1.5
		1 = CT <sup>1</sup>	-0.8	0.2	9	0.7	-0.5	0.3	8	0.7
		0 = TT <sup>1</sup>	-0.6	0.4	0	0.0	0.1	0.5	0	0.0
	1 = CG	2 = CC	-0.8	0.2	71	5.5	-0.9	0.1	60	5.0
		1 = CT	-0.6	0.1	80	6.1	-0.7	0.1	123	10.2
		0 = TT <sup>1</sup>	-0.5	0.2	13	1.0	-0.2	0.2	9	0.7
	0 = GG	2 = CC	-0.7	0.2	54	4.2	-0.7	0.2	33	2.7
		1 = CT	-0.5	0.1	143	11.0	-0.6	0.1	181	15.0
		0 = TT	-0.3	0.1	321	24.7	-0.4	0.1	212	17.5
1 or CG	2 = CC	2 = CC	-0.8	0.2	5	0.4	-0.8	0.1	9	0.7
		1 = CT <sup>1</sup>	-0.7	0.2	3	0.2	-0.3	0.2	1	0.1
		0 = TT <sup>1</sup>	-0.5	0.4	0	0.0	0.2	0.5	0	0.0
	1 = CG	2 = CC	-0.7	0.1	38	2.9	-0.7	0.1	42	3.5
		1 = CT	-0.5	0.1	25	1.9	-0.5	0.1	74	6.1
		0 = TT <sup>1</sup>	-0.3	0.2	7	0.5	0.0	0.2	4	0.3
	0 = GG	2 = CC	-0.5	0.1	62	4.8	-0.6	0.1	23	1.9
		1 = CT	-0.3	0.1	53	4.1	-0.4	0.1	91	7.5
		0 = TT	-0.2	0.0	285	21.9	-0.2	0.1	204	16.9
0 or GG	2 = CC	2 = CC	-0.7	0.2	0	0.0	-0.7	0.1	2	0.2
		1 = CT <sup>1</sup>	-0.5	0.2	1	0.1	-0.1	0.2	1	0.1
		0 = TT <sup>1</sup>	-0.3	0.3	0	0.0	0.4	0.5	0	0.0
	1 = CG	2 = CC	-0.5	0.1	9	0.7	-0.5	0.1	7	0.6
		1 = CT	-0.3	0.1	7	0.5	-0.3	0.1	9	0.7
		0 = TT <sup>1</sup>	-0.2	0.2	6	0.5	0.2	0.2	0	0.0
	0 = GG	2 = CC	-0.4	0.1	17	1.3	-0.4	0.1	5	0.4
		1 = CT	-0.2	0.1	15	1.2	-0.2	0.1	30	2.5
		0 = TT	0.0	0.0	67	5.2	0.0	0.0	63	5.2

<sup>1</sup>These rows include genotypes involving the rare *CAPN1* 316/4751 C-T haplotype. The low number of animals with the C-T haplotype in this study made it difficult to derive an accurate estimate of its effect.

**Table 8.** Power of detecting an allele substitution effect of a given size for each data set<sup>1</sup>

Substitution effect size, SD	GeneSTAR Quality Grade		GeneSTAR Tenderness			Igenity TenderGene		
	QG1	QG2	T1	T2	T3	UoG	CAPN	CAPN
	TG5	M2	CAST	CAPN316	CAPN4751	CAST	316	4751
0.05	0.14	0.12	0.32	0.21	0.23	0.33	0.25	0.34
0.10	0.42	0.33	0.84	0.62	0.69	0.86	0.73	0.87
0.15	0.75	0.63	0.99	0.93	0.96	1.00	0.97	1.00
0.20	0.94	0.86	1.00	0.99	1.00		1.00	
0.25	0.99	0.97		1.00				
0.30	1.00	1.00						

<sup>1</sup>For the GeneSTAR Quality Grade markers, a marbling score SD of ~100 was used; for all of the tenderness markers, a Warner-Bratzler shear force SD of ~1.5 kg was used.

is likely to result. Similar spurious associations can also result from ignoring pedigree. Such associations have no value as tools for genetic improvement.

Finally, an important distinction (which is often overlooked) is that failure to achieve statistical significance should never be interpreted as evidence that an effect is zero. In some cases, the major allele frequency in one or more validation populations may be so high that there is no real opportunity to evaluate the effect of the test. In this case, the failure to find a significant result should not be considered a negative result, but rather it should be considered no result. Given all of these considerations, it is perhaps not surprising that few marker validation studies in cattle have been published. However, validation of the effects of genetic markers in independent populations is likely to be vital to the success of genetic testing technology because some producers may be reluctant to invest in unproven markers.

Validation studies can also serve to generate information that is essential for the process of incorporating DNA tests into the national cattle evaluation. Such information includes the size of allelic substitutions in a range of production environments, allele frequencies in different populations and breeds, and effects of genotypes on nontarget traits. Although there is a tendency to label DNA tests as being associated with one particular trait, markers with a large effect on any one trait are also likely to have correlated effects on other traits because most genes influence a variety of traits (Burrow and Bindon, 2005). As more markers associated with a variety of traits enter the marketplace, it will become increasingly difficult to find independent populations with suitable phenotypes for validation studies. There is a need for the development of large, well-organized, thoroughly phenotyped populations for marker validation studies. The widespread adoption of marker-assisted selection in the industry will likely depend upon the successful integration of marker information into the national cattle evaluation schemes to enable the eventual development of DNA marker-assisted EPD.

In conclusion, tenderness could be markedly improved by selecting for the favorable calpastatin and  $\mu$ -calpain genotypes included in the GeneSTAR Tender-

ness and Igenity *TenderGENE* marker panels. Using the GeneSTAR Quality Grade marker panel may be associated with an increased percentage of USDA Choice or Prime carcasses. Independent, third-party validation of commercial DNA tests provides some assurance to producers that DNA-based tests perform in accordance with the claims of the marketing companies and may help to generate some of the data required to facilitate the integration of marker data into the national cattle evaluation.

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