Enhanced estimates of carcass and meat quality effects for polymorphisms in myostatin and µ-calpain genes[1,2,3]

Gary L. Bennett[4], Richard G. Tait, Jr.[5], Steven D. Shackelford, Tommy L. Wheeler, David A. King, Eduardo Casas[6], and Timothy P. L. Smith

USDA, Agricultural Research Service, Roman L. Hruska US Meat Animal Research Center, Clay Center, NE 68933-0166

ABSTRACT: The objective of this study was to enhance estimates of additive, dominance, and epistatic effects of marker polymorphisms on beef carcass and quality traits. Myostatin (*MSTN*) F94L SNP and the µ-calpain (*CAPN1*) 316 and 4751 SNP haplotype have previously been associated with fat and muscle traits in beef cattle. Multiyear selection in a composite population segregating these polymorphisms increased minor allele (F94L L) and chosen haplotype (*CAPN1* CC and GT) frequencies to intermediate levels resulting in more precise estimates of additive and nonadditive genetic effects. During the 3 yr after selection, 176 steers were evaluated for growth, carcass, meat quality, tenderness (n = 103), and meat color traits. The statistical model included year, age of dam, age of the steer, and genotype in a random animal model. The 9 genotypes (3 *CAPN1* diplotypes × 3 F94L genotypes) affected marbling score, ribeye area, adjusted fat thickness, vision yield grade (all $P < 0.001$), slice shear force ($P = 0.03$), and CIE L^* reflectance ($P = 0.01$). Linear contrasts of the 9 genotypes estimated additive, recessive, and epistatic genetic effects. Significant additive effects of the F94L L allele decreased marbling score, adjusted fat thickness, vision yield grade, and slice shear force; and increased ribeye area and CIE L^* reflectance. The homozygous F94L FF and LL genotypes differed by 1.3 to 1.9 phenotypic SD for most carcass traits and by 0.8 to 0.9 SD for slice shear force and CIE L^* reflectance but carcass weight differed by only 3 kg (0.1 SD). The L allele was partially recessive to F for ribeye area ($P = 0.02$) and the heterozygous FL means tended to be closer to the FF genotype than the LL genotype for other carcass traits but differences from additive were not significant. The *CAPN1* additive × F94L additive effect on slice shear force was the only significant epistatic estimate. The F94L L allele is prevalent in Limousin but nearly absent in other U.S. purebreds. This allele had about half of the effects on birth weight, muscle, and fat traits reported for severe *MSTN* mutations in Belgian Blue and Piedmontese breeds. The interaction between *MSTN* and *CAPN1* genotypes may reflect the strong additive effects of *MSTN* F94L L allele on fat and muscle traits interfering with the phenotypic effect of *CAPN1* genotype on meat tenderness.

Key words: calpain, cattle, epistasis, F94L, myostatin, selection

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[4]Corresponding author: gary.bennett@ars.usda.gov

[5]Current address: Neogen, GeneSeek Operations, 4131 N. 48th St, Lincoln, NE 68504

[6]Current address: USDA, ARS, National Animal Disease Center, 1920 Dayton Ave., Ames, IA 50010

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INTRODUCTION

Some polymorphisms in the myostatin (*MSTN*) gene in cattle reduce functionally effective myostatin resulting in muscle hypertrophy. Before breeders knew what caused muscle hypertrophy and before DNA tests were developed, some breeders selected their cattle for increased muscling (Arthur, 1995). Selection greatly increased frequencies of some *MSTN* alleles in breeds such as Piedmontese and Belgian Blue (Grobet et al., 1997; Kambadur et al., 1997).

Surveys of some European cattle breeds for DNA variants in *MSTN* revealed many polymorphisms (Grobet et al., 1998; Dunner et al., 2003). Among those was F94L, a phenylalanine to leucine substitution at amino acid position 94 in myostatin (Grobet et al., 1998). The leucine allele frequency was high in Limousin and low or absent in many other breeds. Subsequently, this allele was associated with increased muscling and reduced fatness in Limousin crossbreds (Esmailizadeh et al., 2008; Alexander et al., 2009). However, mating designs limited statistical power for estimating dominance effects (Esmailizadeh et al., 2008).

Polymorphisms in μ-calpain (*CAPN1*) are associated with postmortem proteolysis of muscle leading to increased meat tenderness (Page et al., 2004; White et al., 2005; Casas et al., 2006; Robinson et al., 2012). Also, some *MSTN* polymorphisms suppressing production of functional myostatin increase meat tenderness (Wheeler et al., 2001). Casas et al. (2001) identified an interaction of a QTL affecting meat tenderness on BTA 4 with heterozygous *MSTN* and homozygous normal progeny of a Belgian Blue crossbred bull. Epistasis between *MSTN* and *CAPN1* is possible because both genes are thought to affect protein turnover of muscle.

The objective of this study was to estimate additive and nonadditive effects associated with the F94L polymorphism in *MSTN* and SNP 316 and 4751 haplotypes in *CAPN1*. Estimates were enhanced by increasing minor allele frequency of F94L and increasing frequencies of chosen *CAPN1* haplotypes through selection and by using bulls heterozygous for F94L and for *CAPN1*.

MATERIALS AND METHODS

The U.S. Meat Animal Research Center (USMARC) Institutional Animal Care and Use Committee approved the experiment following recommendations by FASS (1999).

Composite Population

A composite cattle population known as MARC I was formed beginning in 1978 and consisted of 0.125 Angus, 0.125 Hereford, 0.25 Braunvieh, 0.25 Charolais, and 0.25 Limousin (Gregory et al., 1991). From 1992 through 1999, the composite was divided into 2 lines: a calving ease selection line and a control line (Bennett, 2008). After completing the selection experiment, cows from both lines were bred to the same bulls and their progeny treated as a single population. During this period from 2000 through 2006, the MARC I population was continued with about 205 calves from 18 sires born each year. Approximately half of sires were replaced each year resulting in the use of 68 bulls selected from within the herd.

Genetic Markers

Selected markers were from 2 genes thought to affect muscles, especially protein turnover and proteolysis. They were the large subunit of micromolar activated calpain (*CAPN1*) and myostatin (*MSTN*). The SNP marker chosen for *MSTN* on BTA 2 was a phenylalanine to leucine substitution at amino acid position 94 in myostatin (F94L; rs110065568, Grobet et al., 1998). Two SNP markers, CAPN1_316 (BTA29; rs17872000) and CAPN1_4751 (BTA29; rs17872050), were used for the *CAPN1* gene located on BTA 29. CAPN1_316 segregates C and G alleles, while CAPN1_4751 segregates C and T alleles. Initial findings associated the C allele of CAPN1_316 with tender beef (Page et al., 2002). Common haplotypes determined from CAPN1_316 and CAPN1_4751 SNP are CC (**CAPN1hCC**), GT (**CAPN1hGT**), and GC (**CAPN1hGC**). A fourth haplotype, CT (**CAPN1hCT**), is rare. White et al. (2005) found the largest difference for 14 d Warner-Bratzler shear force between CAPN1hCC and CAPN1hGT. The most frequent haplotype in that study (CAPN1hGC) was intermediate in tenderness to CAPN1hCC and CAPN1hGT.

Samples of DNA were extracted from blood or semen. Extraction of DNA was done using a Qiagen QIAmp DNA mini blood kit (Qiagen, Valencia, CA). Blood samples were collected in 10 mL syringes with 4% EDTA. Blood was frozen until DNA was extracted. Genotyping was performed using a primer extension method with mass spectrometry-based analysis of the extension products on a MassArray system as suggested by the manufacturer (Sequenom, Inc., San Diego, CA) and described by Stone et al. (2002). When

necessary, genotype assays were repeated to reduce missing genotypes.

Base, Selection, and Evaluation Phases

The experiment was conducted in 3 phases: base, selection, and evaluation. The base phase surveyed live animals and some frozen semen from 4 populations (Angus and 3 composites; MARC I, MARC II, and MARC III) completing the calving ease selection experiment (Bennett, 2008), for *CAPN1* allele and haplotype frequencies. Frequencies of CAPN1hCC, CAPN1hGT, and CAPN1hGC were 0.47, 0.28, and 0.25 for Angus; 0.20, 0.21, and 0.58 for MARC I; 0.20, 0.39, and 0.40 for MARC II; and 0.22, 0.46, and 0.32 for MARC III. Allele L (**F94LaL**) is more frequent than allele F (**F94LaF**) in the Limousin breed but absent or near zero in most other breeds in the United States (Dunner et al., 2003). Limousin makes up 0.25 of MARC I and it was assumed to be the only population segregating F94L. The estimated frequency of F94LaL in MARC I was 20.7% for 363 animals born before 2004. The Limousin specificity was verified later by genotyping Angus (n = 564), MARC II (Angus, Hereford, Gelbvieh, and Simmental; n = 538), and MARC III (Angus, Hereford, Red Poll, and Pinzgauer; n = 747) populations born in 2009, 2010, and 2011 for F94L. All were homozygous for the phenylalanine allele (F94LaF) except for 1 Angus, 1 MARC II, and 2 unrelated MARC III heterozygotes, a frequency less than assumed genotyping error rate. MARC I was chosen for this experiment, because F94LaL was present in the population.

Selection was applied from 2004 through 2006 with the goal of increasing frequencies of F94LaL, CAPN1hCC, and CAPN1hGT to 0.5 and eliminating CAPN1hGC. Calves were bled before weaning, the DNA was extracted, and then genotyped. Marker genotypes were used to select replacement bulls and heifers soon after weaning. Selection of replacement animals in this phase was based on the presence of F94LaL, CAPN1hCC, and CAPN1hGT and absence of CAPN1hGC.

The evaluation phase (birth years 2007, 2008, and 2009) increased the number of animals evaluated for carcass traits. This phase also used bulls heterozygous for both F94L and *CAPN1* if available. These bulls increase the number of within sire comparisons across any combination of progeny genotypes. A heterozygous bull can sire progeny with heterozygous or either homozygous genotype. Thirty bulls sired calves in the evaluation phase. Bulls heterozygous for CAPN1hCC/CAPN1hGT (n = 15) sired 70% of calves. Bulls heterozygous for F94L (n = 23) sired 87% of calves. Bulls heterozygous for both *CAPN1* and F94L (n = 10) sired 59% of calves.

Blood samples from spring-born progeny were collected before weaning and genotyped. Calves with incomplete genotypes were removed from the experiment. Replacement bulls were randomly sampled within sire from among males heterozygous for both F94L and the chosen *CAPN1* haplotypes. The remaining males were castrated by banding soon after weaning and genotyping. Steers consumed corn and corn silage-based diets until harvest. Weights were taken at birth (mean date = April 13), at weaning (mean age = 159 d, SD = 18 d), and as yearlings (mean age = 344 d, SD = 22 d).

All experimental steers were harvested on a single day each year at a commercial abattoir at an average age of 487 d. In 2010 (born 2009), 29 steers were removed from the experiment based on either having unselected haplotypes (CAPN1hGC or CAPN1hCT) or having genotypes in common with many steers (reduced at random). Carcasses were weighed hot, electrically-stimulated, and chilled using the commercial facility's proprietary system. At 36 h postmortem, carcasses were ribbed between the 12th and 13th ribs and an image analysis based (VBG2000) grading system (Shackelford et al., 2003) assessed adjusted fat thickness, ribeye area, USDA marbling score, CIE L^* of the longissimus muscle, and calculated vision yield grade. A longissimus steak from the 13th rib region was returned to USMARC to evaluate slice shear force at 14 d postmortem (Shackelford et al., 1999).

Statistical Analysis

Either trait measurements or logarithms of measurements (marbling score; slice shear force) were analyzed with a mixed model using MTDFREML (Boldman et al., 1995). The model was:

$$Y_{i,j,k,l} = \mu + Year_i + Aod5_j + Genotype_k + b \times Age_{i,j,k,l} + a_{i,j,k,l} + e_{i,j,k,l}$$

where $Y_{i,j,k,l}$ is the observation or its logarithm for the *i, j, k, l-th* animal, μ is the mean, $Year_i$ is birth year 2007, 2008, or 2009, $Aod5_j$ is age of dam (2, 3, 4, or ≥ 5 yr), b is a linear regression coefficient on the *i, j, k, l-th* animal's age ($Age_{i,j,k,l}$) in days, $Genotype_k$ is 1 of the 9 combinations of 3 *CAPN1* diplotypes and 3 F94L genotypes, $a_{i,j,k,l}$ is the additive polygenic animal effect, and $e_{i,j,k,l}$ is of the residual effect of the *i, j,*

k, l-th observation. Covariances of polygenic effects were assumed proportional to the pedigree relationship matrix. Residual effects were assumed independent with constant variance. The pedigree used to calculate relationships included more than 6,600 animals. Heritabilities were constrained between 0.20 and 0.70 because of few observations and imprecise genetic variance estimates. Similar ranges in heritabilities for these traits were estimated from larger, related populations (Gregory et al., 1994; Bennett and Gregory, 1996). Skewed distributions of marbling scores and meat tenderness values were transformed using base 10 logarithms to determine *P* values but reported means and contrasts are from analyses of untransformed data.

A $P < 0.10$ for the genotype effect (9 genotypes) was used as a guideline for then calculating linear contrasts for additive, dominance, and epistasis effects associated with *CAPN1* haplotypes and F94L alleles similar to Tait et al. (2016). Linear contrast coefficients used to estimate genetic effects (Table 1) which were divided by their SE to determine significance based on a *t*-test. Only testing genetic effect contrasts after meeting an overall genotype probability test (e.g., $P < 0.10$ guideline) protects against probability inflation due to multiple testing. A significance level of $P < 0.05$ was used for individual contrasts.

RESULTS AND DISCUSSION

Frequencies of chosen *CAPN1* haplotypes and F94LaL changed during the 3 phases of the experiment (Fig. 1). The frequency of F94LaL and CAPN1hCC approached 0.5 during the evaluation phase. The combined frequencies of the 2 selected *CAPN1* haplotypes were about 0.4 during the base phase and increased to 0.75 during the evaluation phase (Fig. 1). About 40% of animals still had at least one CAPN1hGC haplotype during the evaluation phase. Because CAPN1hCC was more frequent than CAPN1hGT, analyses of all traits except slice shear force used CAPN1hCC and all other haplotypes (CAPN1hNN) instead of CAPN1hGT specifically. Only 103 animals with unambiguous diplotypes consisting of only CAPN1hGT and (or) CAPN1hCC haplotypes were used to analyze slice shear force. This method of addressing the CAPN1hGC haplotypes was chosen for slice shear force because a direct effect of *CAPN1* on this trait was expected. This method should have no bias and a straight forward interpretation of CAPN1hCC and CAPN1hGT associations but does result in fewer animals and less power. The CAPN1hNN method was used for all other traits because *CAPN1* effects, if any, are expected to be less direct. The CAPN1hNN method maximized the number of steers that could be used to estimate F94L effects for traits other than slice shear force while still accounting for the possibility of *CAPN1* influence.

Table 2 shows the combinations of *CAPN1* diplotypes and F94L genotypes for the 176 harvested steers and the 103 used in slice shear force analyses. Table 3 characterizes the opportunity for

Table 1. Linear contrast coefficients used to estimate additive, dominance, and epistasis effects for μ-calpain (*CAPN1*) haplotype and F94L SNP

Genotype mean[1]		F94L[2]		*CAPN1*[2]		F94L × *CAPN1*[3]			
F94L	*CAPN1*	A	D	A	D	AA	AD	DA	DD
FF	NN-NN	−1	−1	−1	−1	1	1	1	1
FF	CC-NN	−1	−1	0	2	0	−2	0	−2
FF	CC-CC	−1	−1	1	−1	−1	1	−1	1
FL	NN-NN	0	2	−1	−1	0	0	−2	−2
FL	CC-NN	0	2	0	2	0	0	0	4
FL	CC-CC	0	2	1	−1	0	0	2	−2
LL	NN-NN	1	−1	−1	−1	−1	−1	1	1
LL	CC-NN	1	−1	0	2	0	2	0	−2
LL	CC-CC	1	−1	1	−1	1	−1	−1	1
	Divisor[4]	6	6	6	6	1	2	2	4

[1]Estimated genotype means are identified by the 9 combinations of 3 *MSTN* F94L genotypes and 3 μ-calpain (*CAPN1*) diplotypes. F94L genotypes are designated by FF (homozygous F94LaF), FL (heterozygotes), and LL (homozygous F94LaL). *CAPN1* diplotypes are designated NN-NN (homozygous CAPN1hNN), CC-NN (heterozygotes) and CC-CC (homozygous CAPN1hCC).

[2]Linear contrast coefficients multiplied by genotype means to estimate F94LaL and CAPNhCC haplotype additive (A) and dominance (D) effects.

[3]Linear contrast coefficients for 2-factor epistatic effects identified with 2 letters. The first letter is the F94LaL effect and the second letter is the CAPN1hCC haplotype effect, e.g., AD is additive F94L × *CAPN1* dominance epistatic effect.

[4]Actual coefficients used were the whole numbers in table divided by this number.

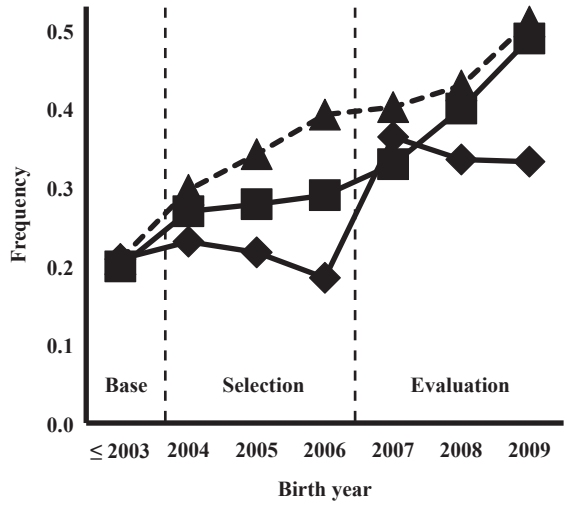

Figure 1. Frequencies for MSTN F94L allele L (▲) and μ-calpain 316–4751 haplotypes CC (■) and GT (♦) by birth year. Base, selection, and evaluation phases of the experiment are identified by vertical dashed lines.

Table 2. Number of harvested MARC I steers by genotype

CAPN1 diplotype[1]	F94L genotype[2]			Total
	FF	FL	LL	
CAPN1hNN, CAPN1hNN	26	26	9	61
CAPN1hGT, CAPN1hGT	*9*	*12*	*2*	*23*
CAPN1hCC, CAPN1hNN	24	40	16	80
CAPN1hCC, CAPN1hGT	*17*	*18*	*10*	*45*
CAPN1hCC, CAPN1hCC	8	17	10	35
Total	58	83	35	176

[1]Diplotypes composed of μ-Calpain (*CAPN1*) haplotypes. Any haplotype other than CAPN1hCC is represented by CAPN1hNN and these animals were used for analyses of most traits. Only animals with diplotypes consisting of CAPN1hGT and CAPN1hCC were used to analyze slice shear force (n = 103) and are a subset of the 176 steers and indicated by italics.

[2]*MSTN* F94L homozygous F94LaF (FF), heterozygous (FL), and homozygous F94LaL (LL) genotypes.

Table 3. Characterization of potential for within sire comparisons among genotypes

Progeny distribution measures for 28 sires	Value
Median number of progeny per sire	4.5
Sires with 3 progeny F94L genotypes[1]	12
Average F94L progeny genotypes per sire	2.04
Sires with 3 progeny *CAPN1* diplotypes[2]	7
Average *CAPN1* progeny diplotypes per sire	2.11
Sires with 7 to 9 *CAPN1* × F94L progeny genotypes	4
Sires with 1 to 3 *CAPN1* × F94L progeny genotypes	16
Median *CAPN1* × *MSTN* progeny genotypes per sire	3

[1]Myostatin (*MSTN*) F94L genotypes were homozygous F94LaF, heterozygous F94L, and homozygous F94LaL.

[2]μ-Calpain (*CAPN1*) 316–4751 diplotypes were homozygous CAPN1hCC, the heterozygotes, and homozygous CAPN1hNN.

Table 4. Averages and SD of unadjusted measurements on 176 harvested steers

Trait	Average	SD
Birth weight, kg	41.0	5.5
Weaning weight, kg	202	25
Yearling weight, kg	409	44
Final weight, kg	604	55
Hot carcass weight, kg	376	37
Marbling score[1]	329	38
Ribeye area, cm[2]	94.0	9.2
Adjusted fat thickness, mm	9.3	3.6
Vision yield grade[2]	2.26	0.73
Slice shear force[3], kg	14.8	4.0
CIE L^* [4]	35.6	2.0

[1]300 = Slight[00]; 400 = Small[00] (USDA, 1997).

[2]Prediction of USDA Yield Grade. Smaller numbers indicate greater yield of boneless, closely trimmed retail cuts.

[3]Values for 103 steers having only diplotypes consisting of CAPN1hCC and (or) CAPN1hGT and analyzed for slice shear force.

[4]CIE L^* measure of lightness. Greater values indicate lighter lean color.

increasing power through within sire comparisons across genotypes. Median values for 28 sires used were 4.5 progeny distributed among 3 of the 9 possible genotypes (Table 3). Four sires had progeny in 7, 8, or 9 genotype classes making especially strong contributions to increasing power. For example, the 2 steers in the smallest *CAPN1* × *MSTN* combination for slice shear force had 18 half-sibs among 7 or the other 8 *CAPN1* × *MSTN* combinations.

Averages for steer traits, their estimated heritabilities, SD, and phenotypic SD are shown in Tables 4 and 5. The P-values for genotypes (8 df) were not significant for any weights from birth through harvest (Table 5). Genotype effects were significant ($P < 0.05$) for all carcass and meat traits except hot carcass weight. Estimated genotype and diplotype means for all traits are shown in Table 6.

Linear contrasts for genetic effects were estimated for significant carcass and meat traits (Table 7). Additive effects of *MSTN* F94L were significant ($P < 0.01$) for all these traits. Reduced fat thickness, larger ribeye area, and better yield grade were associated with F94LaL. It was also associated with lower marbling scores, more tender meat, and lighter meat color. The F94LaL was partially recessive to F94LaF for ribeye area resulting in the heterozygote being closer to the F94LaF homozygote (Tables 6 and 7). The estimated differences of homozygous F94LaF, heterozygous, and homozygous F94LaL on traits with significant additive and dominance effects are shown in Fig. 2. Differences are standardized by subtracting the average of

Table 5. Heritability and phenotypic SD estimates and *P*-values for sources of variation

Trait	Year	Dam age	Calf age[1]	Genotype	h^2	σ_P
Birth weight, kg	0.10	0.001	0.09	0.38	0.68	5.3
Weaning weight, kg	0.91	<0.001	<0.001	0.73	0.30	19.0
Yearling weight, kg	<0.001	<0.001	<0.001	0.99	0.31	24.4
Final weight, kg	0.005	0.001	<0.001	0.85	0.50	48.5
Hot carcass weight, kg	<0.001	0.001	<0.001	0.92	0.42	31.4
Marbling score[2,3]	0.13	0.60	0.53	<0.001	0.47	34
Ribeye area, cm^2	0.004	0.04	0.16	<0.001	0.39	7.1
Adjusted fat thickness, mm	0.15	0.61	0.23	<0.001	0.51	3.2
Vision yield grade[4]	0.22	0.35	0.10	<0.001	0.52	0.62
Slice shear force[3], kg	0.08	0.94	0.34	0.03	0.20[5]	4.0
CIE *L** reflectance[6]	0.03	0.86	0.05	0.01	0.44	1.9

[1]Julian birthday linear covariate was used for all traits and is a proxy for age for all traits (except birth weight) because a single harvest date was used each year.

[2]300 = Slight[00]; 400 = Small[00] (USDA, 1997).

[3]Logarithm (base 10) values of traits were analyzed.

[4]Prediction of USDA Yield Grade. Smaller numbers indicate greater yield of boneless, closely trimmed retail cuts.

[5]Constrained to $0.20 \leq h^2 \leq 0.70$.

[6]CIE *L** measure of lightness. Greater values mean lighter lean color.

Table 6. Means of traits by myostatin F94L genotypes and μ-calpain (*CAPN1*) diplotypes

Trait	F94L[1]			*CAPN1*[2]			SED[3]	
	FF	FL	LL	NN-NN	NN-CC	CC-CC	SEDhom	SEDhet
Birth weight, kg	40.7	41.4	43.3	41.7	41.3	42.4	1.2	1.0
Weaning weight, kg	202	199	206	204	202	201	4.6	3.9
Yearling weight, kg	409	410	412	408	409	413	8.5	7.1
Final weight, kg	608	603	598	599	604	607	11.6	9.7
Hot carcass weight, kg	375	375	378	374	377	377	7.6	6.3
Marbling score[4]	345	329	294	325	327	316	8.2	6.8
Ribeye area, cm^2	89	93	103	95	95	95	1.7	1.4
Adjusted fat thickness, mm	10.5	9.1	6.4	8.9	8.9	8.3	0.8	0.6
Vision Yield Grade[5]	2.59	2.26	1.57	2.18	2.18	2.05	0.15	0.12
Slice shear force, kg	16.2	14.5	12.8	15.1	13.7	14.8	0.96	0.81
CIE *L** reflectance[6]	34.65	35.58	36.20	35.40	35.58	35.44	0.45	0.38

[1]*MSTN* F94L homozygous F94LaF (FF), heterozygous (FL), and homozygous F94LaL (LL) genotypes.

[2]*CAPN1* diplotypes are designated NN-NN (homozygous CAPN1hNN), CC-NN (heterozygotes) and CC-CC (homozygous CAPN1hCC).

[3]Approximate within gene SED for the difference between homozygotes (SEDhom) and between the heterozygote and either of the homozygotes (SEDhet).

[4]300 = Slight[00]; 400 = Small[00] (USDA, 1997).

[5]USDA vision yield grade. Smaller numbers indicate greater yield of boneless, closely trimmed retail cuts.

[6]CIE *L** measure of lightness. Lighter lean color results in greater values.

the homozygotes and dividing by phenotypic SD. Although genotype effect (8 df) was not significant in this study ($P = 0.38$; Table 5), birth weight is also shown because it could affect use of F94LaL in mating systems, approached significance ($P = 0.06$) in heifers that were sibs to these steers (Cushman et al., 2015), and has shown significant increases in other homozygous *MSTN* mutations (e.g., Casas et al., 2004).

Multiple variants in *MSTN* that decrease functional activity and cause muscular hypertrophy in cattle have been found (Dunner et al., 2003). An 11 base pair deletion in the Belgian Blue breed causes a frame shift in the translation frame that prevents translation of the active signaling domain of the protein. In Piedmontese, there is a single base change that eliminates a proteolytic self-cleavage site immediately proximal to the signaling domain that prevents process to the active form. These 2 highly disruptive variants contrast with the relatively minor substitution of an aliphatic side chain (leucine) for an aromatic (phenylalanine) in the

Table 7. Estimated marker associated additive and nonadditive effects for traits with overall $P < 0.10$ for genotype

Marker effect[1]	Marbling score[2]	Ribeye area, cm[2]	Adjusted fat thickness, mm	Vision Yield Grade	Slice shear force[2], kg	*L** reflectance
	Value ± SE	Value ± SE	Value ± SE	Value ± SE	Value ± SE	Value ± SE
F94L A	−25.4*** ± 3.9	6.6*** ± 0.8	−2.06*** ± 0.36	−0.57*** ± 0.07	−1.7** ± 0.7	0.78*** ± 0.21
F94L D	9.4 ± 5.6	−2.7* ± 1.2	0.61 ± 0.52	0.18 ± 0.10	0.0 ± 0.9	0.16 ± 0.30
CAPN1 A	−4.7 ± 4.3	0.3 ± 0.9	−0.32 ± 0.40	0.07 ± 0.08	−0.1 ± 0.7	0.02 ± 0.24
CAPN1 D	5.8 ± 5.5	0.0 ± 1.1	0.30 ± 0.51	0.07 ± 0.10	−1.3 ± 0.9	0.16 ± 0.30
A × A	13.4 ± 20.4	−2.3 ± 4.3	0.61 ± 1.90	0.06 ± 0.37	7.3* ± 3.7	0.49 ± 1.12
A × D	9.2 ± 15.0	5.2 ± 3.1	−1.03 ± 1.40	−0.23 ± 0.27	−0.8 ± 2.5	0.80 ± 0.82
D × A	13.2 ± 14.4	−2.6 ± 3.0	−0.01 ± 1.37	0.21 ± 0.26	−0.3 ± 2.4	0.96 ± 0.79
D × D	4.1 ± 10.3	2.3 ± 2.2	1.06 ± 1.02	0.05 ± 0.19	−0.1 ± 1.7	−0.51 ± 0.56

[1]Epistatic effects are listed as myostatin F94L effect × μ-Calpain (*CAPN1*) haplotype effect. Linear contrast coefficient used to estimate additive (A), dominance (D), and epistatic (A × A, D × A, A × D, D × D) effects are shown in Table 1.

[2]Means and SE estimates from actual values. Significance determined from analyses of logarithms of data.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

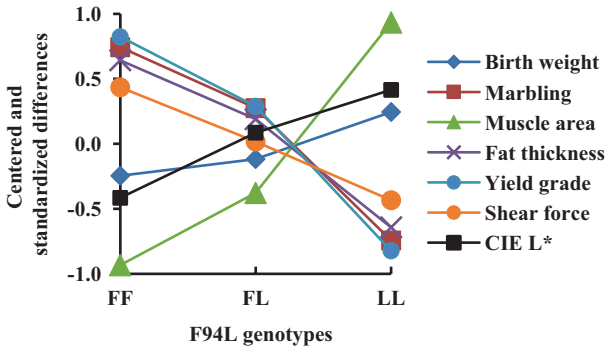

Figure 2. Means for *MSTN* F94L genotypes divided by their phenotypic SD and deviated from the average of F94LaF (FF) and F94LaL (LL) homozygotes. All differences between divergent homozygotes are significant except birth weight. Heterozygotes were different from the average of homozygotes for ribeye muscle area ($P < 0.05$). Birth weight is included for comparison with previous literature reports of significant differences.

propeptide domain resulting from the F94L variant, because both are nonpolar residues. However, it is possible this substitution affects protein folding, stability, or trafficking of the myostatin protein with the primary evidence for this being the effect on muscle growth in cattle (Alexander et al., 2007). Differences between F94L homozygote means in this study ranged from 1.3 to 1.9 phenotypic SD for fat and muscle traits. Most carcass traits show a nonsignificant tendency for the heterozygote to be partially recessive to the F94LaF allele (the mean being closer to the homozygous F94LaF mean than the homozygous F94LaL mean), although the effect did reach significance for ribeye area. Esmailizadeh et al. (2008) reported on Limousin-Jersey backcross families in Australia and New Zealand. They found no significant effects of the F94LaL SNP on birth and live weights. Additive and dominance effects on Longissimus muscle area were significant but not as large as the additive effects in this study. Several measures of fatness were also decreased, and meat weights increased. Alexander et al. (2009) found increased muscle area and reduced marbling in a Wagyu-Limousin F_2 family.

Differences in birth weight, fat, and muscle traits between homozygotes for some of the severe *MSTN* variants estimated in 2 other experiments exceed the values for F94L estimated in this experiment. Casas et al. (2004) compared homozygous active and inactive F_2 progeny from Belgian Blue $F_1 \times F_1$ matings in the same location and under similar management as this experiment. Short et al. (2002) compared F2 Piedmontese under similar management but in a different location. Both experiments found significant differences between divergent homozygotes for birth weight, ribeye area, marbling score, fat thickness, and yield grade. The average percentage differences from the active *MSTN* homozygotes were 15%, 32%, −32%, −62%, and −78%, respectively, compared with 7%, 15%, −14%, −39%, and −38% for F94L in this experiment. The effects of the F94L mutation were about half the percentage differences of the Belgian Blue and Piedmontese mutations.

The *CAPN1* haplotypes had no significant additive or dominance associations with any measured trait including meat tenderness. A significant *CAPN1* additive by F94L additive epistatic effect is illustrated in Fig. 3. One way of characterizing this epistasis is that CAPN1hCC reduced slice shear force (increased tenderness) in animals homozygous for the common F94LaF allele, had no effect in F94L heterozygotes, and decreased tenderness in F94LaL homozygotes. Increased tenderness is often observed in meat from animals with heterozygous

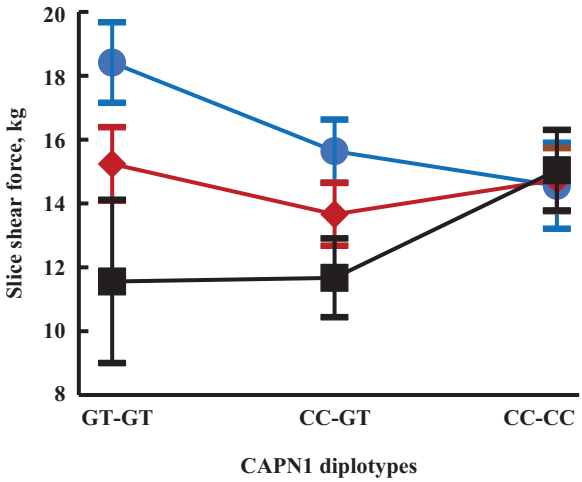

Figure 3. *MSTN* F94L × μ-calpain genotypic means for slice shear force. F94L homozygous F94LaF, heterozygous, and homozygous F94LaL genotypes are designated by FF, FL, and LL, respectively. *CAPN1* diplotypes are designated GT-GT (homozygous CAPN1hGT), CC-GT (heterozygotes) and CC-CC (homozygous CAPN1hCC). The additive effect is significant for F94L ($P < 0.01$) and the additive F94L × additive μ-calpain effect is significant ($P < 0.05$). Variation is shown as ±1 SEM.

and homozygous *MSTN* mutations resulting in nonfunctional myostatin (e.g., Wheeler et al., 2001). Because few animals are homozygous CAPN1hCC in most common populations surveyed (White et al., 2005), F94LaL would be associated with increased tenderness in most populations using the epistatic estimates. Frequency of CAPN1hCC was increased in 2 similar experiments. In an Angus population (Tait et al., 2014a), the estimated additive effect of CAPN1aCC (compared to CAPN1aGT) on slice shear force was −1.05 ± 0.25 kg and in the composite MARC III population (Tait et al., 2014b) was −1.15 ± 0.48 kg. Using only estimated means for homozygous F94LaF, the equivalent additive estimate was −1.93 ± 0.97 kg in the current study. The usual relationship between *CAPN1* haplotypes and meat tenderness appears to be disrupted by the F94LaL allele. Previous QTL discovery in progeny of an F1 Piedmontese sire and an F1 Belgian Blue sire heterozygous for active and inactive myostatin found interactions of the *MSTN* variants with other QTL located on BTA 4 (Casas et al., 2001) and BTA 5 (Casas et al., 2000) for meat tenderness.

Research using heifer half-sibs from this population showed delayed age of puberty due to F94LaL alleles (Cushman et al., 2015). However, conception was not reduced or delayed in this population and management system. This moderate form of *MSTN* mutation with high frequency in the Limousin breed shows the potential for variation among the effects of mutations in some genes. In this case, the resulting increase in lean meat yield, moderate birth weight increase, and limited effect on heifer conception is a useful resource for improving efficiency of lean beef production in conventional production systems. Because F94L effects are mostly additive, 0, 1, or 2 copies of F94LaL can be used to create 3 product types with increasing lean meat yield and decreasing marbling. Terminal cross bulls with 1 or 2 copies of F94LaL mated to cows with 0 copies would produce 0 and 1 copy progeny (1 copy bulls) or 1 copy progeny (2 copy bulls). Based on the epistatic estimates for slice shear force, *CAPN1* selection would be less effective for progeny with 1 copy but effective for progeny with 0 copies.

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