

Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle¹

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ABSTRACT: Calpastatin (CAST) is a naturally occurring protein that inhibits the normal tenderization of meat as it ages postmortem. A SNP was identified in the CAST gene (a G to C substitution) and genotyped on crossbred commercially fed heifers (n = 163), steers (n = 226), and bulls (n = 61) from beef feedlots, and steers (n = 178) from a University of Guelph feeding trial. The association of the CAST SNP with carcass and meat quality traits was studied. Carcass traits included fat, lean, and bone yield; grade fat; LM area; and HCW. Meat quality traits included marbling grade; i.m. fat content of LM; tenderness evaluation of LM (Warner-Bratzler shear force) at 2, 7, 14, and 21 d of postmortem aging; and tenderness evaluation of semitendinosus muscle at 7 d of postmortem aging. The mixed model used in the analyses included fixed effects of CAST genotype, sex, slaughter group, and breed composition (linear covariate); sire was a random effect. For the analysis of shear force, i.m. fat content of LM was also included in the model as a linear covariate. Shear force measures were analyzed within days of postmortem aging and by repeated measures analysis. The CAST SNP allele C was more frequent (63%) in the crossbred

population than allele G. The CAST SNP was associated with shear force across days of postmortem aging ($P = 0.005$); genotype CC yielded beef that was more tender than GG ($-0.32 \text{ kg} \pm 0.13$), and CG had intermediate tenderness. The corresponding average allele substitution effect (G to C substitution) was also highly significant ($-0.15 \pm 0.05 \text{ kg}$, $P = 0.002$). A lower percentage of unacceptably tough steaks (shear force $>5.7 \text{ kg}$) at 2 and 7 d postmortem was associated with an increasing number of C alleles ($P \leq 0.05$). At 7 d postmortem, the percentage of unacceptably tough steaks decreased by 24 and 35%, respectively, for animals carrying 1 and 2 copies of the C allele relative to animals with no C alleles. However, genotype CC had a greater fat yield ($+1.44 \pm 0.56\%$; $P = 0.037$) than genotype GG, with a corresponding allele substitution effect of $0.67 \pm 0.27\%$ ($P = 0.015$). Therefore, the CAST SNP allele C was associated with increased LM tenderness across days of postmortem aging and, importantly for the beef industry, had a significant reduction in the percentage of steaks rated unacceptably tough by consumers based on an assumed threshold level.

Key words: beef breed, calpastatin gene, carcass trait, meat quality, single nucleotide polymorphism, tenderness

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INTRODUCTION

Meat tenderness is an important issue in beef cattle production because it has a major impact on consumer satisfaction. Consumers consider tenderness to be the

single most important component of meat quality (Miller et al., 1995).

The physiological change in muscle structure during the postmortem period is complex (Koochmaraie, 1994). The calpain/calpastatin system is an endogenous, calcium-dependent proteinase system, theorized to mediate the proteolysis of key myofibrillar proteins during postmortem storage of carcass and cuts of meat at refrigerated temperatures (Koochmaraie et al., 1995b). Calpain is responsible for the breakdown of myofibrillar proteins, which are closely related to meat tenderness (Wheeler and Koochmaraie, 1994). Calpastatin (CAST) inhibits μ - and m-calpain activity and, therefore, regulates postmortem proteolysis. Increased postmortem CAST activity has been correlated with reduced meat tenderness (e.g., Koochmaraie et al., 1995a; Pringle et al., 1997).

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The CAST gene, mapped to BTA 7 (Bishop et al., 1993), is considered a candidate gene for beef tenderness. Initial studies did not detect significant association of CAST polymorphisms with tenderness (e.g., Lonergan et al., 1995; Chung et al., 1999). However, in both studies the sample sizes were small, which may have contributed to the failure to identify significant associations between the CAST gene polymorphisms and meat tenderness.

More recently, genetic tests for meat tenderness in beef, which utilize genetic polymorphisms in the CAST and/or Calpain genes, have been made available by private companies. Examples are the IGENITY TenderGENE test (Merial Ltd., Atlanta, GA) and the GeneSTAR Tenderness 2 test (Genetic Solutions Pty. Ltd., Albion, Australia).

The objective of this study was to assess the association of a newly discovered SNP in the CAST gene with meat tenderness and other important meat and carcass quality traits in a large sample of crossbred beef cattle.

MATERIALS AND METHODS

Cattle

A total of 628 animals were genotyped, including commercially fed heifers (n = 163), steers (n = 226), and bulls (n = 61) from beef feedlots, and steers (n = 178) from a University of Guelph feeding trial in Rockwood, Ontario. Commercial cattle originated from 16 feedlots. The 2 sources of cattle were identified as Commercial and Rockwood, respectively.

Animals were crossbred with breed composition formed by several breeds. The major contributing breeds were Angus, Limousin, Charolais, and Simmental. The average contribution of these 4 breeds to the breed composition of animals having any non-zero fraction of the mentioned breeds was 0.46, 0.50, 0.50, and 0.50 for Angus, Limousin, Charolais, and Simmental, respectively, for Commercial cattle and was 0.51, 0.59, 0.53, and 0.41 for Rockwood cattle. Given the primary use of purebred sires, Commercial cattle were more representative of first generation crossbreds than Rockwood cattle.

Rockwood and Commercial cattle represented AI breeding as well as some herd bulls. Animals were finished in commercial feedlots and marketed based on the feedlot operators' definition of finish, which was a visual appraisal of fat cover and weight. Resulting HCW and grade fat levels are listed in Table 1.

DNA Isolation

The sources of DNA were frozen steaks stored in the Meat Science Laboratory at University of Guelph. The DNA was isolated from the meat sample by the standard phenol/chloroform method (Sambrook et al., 1989). The cell lysis buffer was modified as a 0.8 M concentration of urea, including 0.8 M urea, 2% SDS, 100 mM Tris-HCl, 200 mM NaCl, and 2 mM EDTA, pH 7.5.

Table 1. Number of phenotypic records for carcass and meat quality traits with corresponding means and SD

Trait ¹	Records	Mean	SD
FATYL, %	602	25.4	5.25
LEANYL, %	602	55.1	4.70
BONEYL, %	602	19.5	2.00
GFAT, mm	623	9.9	3.91
LMA, cm ²	601	86.5	13.72
HCW, kg	621	334.2	41.54
SFL2, kg	466	5.4	1.69
SFL7, kg	628	5.0	1.48
SFL14, kg	627	4.3	1.33
SFL21, kg	624	3.8	1.03
SFLavg, kg	465	4.5	1.03
SFS7, kg	620	5.0	0.97
CF, %	624	3.9	1.66
MG A, ² %	75	12.2	
MG AA, ² %	355	57.8	
MG AAA, ² %	184	30.0	

¹Fat (FATYL), lean (LEANYL), and bone (BONEYL) yield, grade fat (GFAT), LM area (LMA), HCW, LM shear force (SFL) at 2, 7, 14, and 21 d of postmortem aging, and average shear force across d of aging (SFLavg), semitendinosus muscle shear force (SFS) at 7 d of postmortem aging, LM chemical fat (CF), and marbling grade (MG).

²Observed frequency of the marbling grades (A = traces, AA = slight, and AAA = small presence of marbling or greater).

Primer Design and Sequencing of Amplified DNA Fragments

One pair of primers—forward: 5'-cct cga ctg cgt acc aat tcc gaa gta aag cca aag gaa ca-3' and reverse: - 5'-att tct ctg atg gtg gct gct cac t-3'—was designed based on a partial genomic DNA sequence of the bovine CAST gene (Accession number AY008267), using the software Primer 3 (<http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi>). To simplify the sequencing procedures when performing many reactions for different genes, a common tail was also added to the forward primer: 5'-cct cga ctg cgt acc aat tcc-3'.

The PCR master mix was 6.71 µL of distilled H₂O, 1.0 µL of 10× PCR buffer II, 0.73 µL of 25 mM MgCl₂ solution, 0.6 µL of 10 mM dNTPs Solution, 0.13 µL of forward primer (20 pmol/µL), 0.13 µL of reverse primer (20 pmol/µL), 0.1 µL of AmpliTaq Gold (5 U/µL; all from Applied Biosystems, Foster City, CA), and 0.6 µL of DNA template (50 ng/µL) in a 10-µL reaction solution. The PCR cycling condition was 95°C (10.0 min) for 1 cycle; then 94°C (30 s), 69 to 62°C (30 s) and 72°C (30 s) for 8 cycles; then 94°C (30 s), 61°C (30 s), and 72°C (30 s) for 27 cycles; and finally 72°C (5.0 min) for 1 cycle, maintaining 4°C thereafter. The PCR reactions were performed on the GenAmp PCR System 9700 (Applied Biosystems). The corresponding PCR products were 523 bp. Sequencing of the PCR products revealed a G/C transversion in the products, resulting in the presence/absence of a *RsaI* restriction site at the position 257 bp with recognizing sequence gt/ac. Sequencing was performed on the ABI PRISM 377 DNA sequencer (Applied Biosystems) by the Laboratory Service Division, University of Guelph.

PCR-RFLP Genotyping

Individual PCR-RFLP genotypes were distinguished for polymorphisms of CAST *RsaI*. The PCR products were digested by the restriction endonuclease. Digestion was conducted at 37°C for 4 h and in a 10- μ L reaction solution including 2.7 μ L of distilled H₂O, 1.0 μ L of specific NEBuffer, 0.3 μ L (3 units) of restriction endonucleases, and 6 μ L of PCR product solution. The DNA fragments from the digested PCR products were separated by 2.0%-agarose (Fisher Scientific Ltd., Nepean, Ontario, Canada) gel containing ethidium bromide (0.4 μ g/mL). Electrophoresis was performed in 1 \times TBE buffer including 108 g of Tris, 55 g of boric acid, and 40 mL of 0.5 M EDTA in 1,000 mL of 10 \times concentrated stock solution, pH 8.0, containing ethidium bromide (3 μ L of 10 mg EB/mL per 100 mL of gel solution) under 120 V for about 45 min. Genotype for each individual was read under ultraviolet light, using Molecular Analyst Software (BioRad Laboratories, Molecular Bioscience Group, Hercules, CA).

Phenotypic Information

Information on tenderness (shear force) of LM at 2 (**SFL2**), 7 (**SFL7**), 14 (**SFL14**), and 21 (**SFL21**) d post-mortem and of semitendinosus muscle at 7 (**SFS7**) d postmortem, chemical fat (**CF**), grade fat (**GFAT**), marbling grade (**MG**), LM area (**LMA**), percentage of lean (**LEANYL**), fat (**FATYL**), and bone (**BONEYL**) yield, and HCW were available on most of the 628 genotyped animals.

Warner-Bratzler peak shear force measurements (kg) were used as an objective method of assessing tenderness (Shackelford et al., 1999). The GFAT was the back-fat thickness measurement taken at the 12th and 13th rib interface. The LMA was the measure of the LM area at the 12th and 13th rib interface using a tracing of the muscle. The CF was the chemical analysis on a core meat sample that determined the percentage of i.m. fat in the LM. The LEANYL, FATYL, and BONEYL were determined by dissection of a 4-bone rib section.

Marbling grade was the grade used for grading in Canada (A, AA, AAA, Prime) with most carcasses falling in 1 of the first 3 categories. The assessment of marbling is based on the average amount, size, and distribution of fat particles or deposits in the LM. Canadian beef carcass grading utilizes only 4 of the 9 recognized levels of marbling from the USDA marbling standards with the following minimums for each category: traces (A), slight (AA), small (AAA), and slightly abundant (Prime) marbling (CBGA, 2005). Because only a few carcasses were classified as Prime, those carcasses were combined with AAA carcasses for the analyses. A complete description of carcass measures available and methods used are given in Laborde et al. (2001).

Cattle were slaughtered at the slaughter plant of Better Beef Ltd. in Guelph. Standardized carcass and meat quality measures were made either at the Univer-

sity of Guelph Meat Science Laboratory (FATYL, LEANYL, BONEYL, CF, and tenderness evaluation of LM and semitendinosus muscle) or at the Better Beef plant (GFAT, HCW, LMA, and MG) by University of Guelph meat scientists. Commercial cattle were slaughtered from 1998 to 2000, and Rockwood cattle were slaughtered in 1998.

The genotypes of all animals were used to determine the allele frequencies. For the study of the association of CAST SNP with carcass and meat quality traits, only animals with required phenotypic information were used. The resulting number of records ranged from 466 for SFL2 to 628 for SFL7. Table 1 gives the number of records, means, and SD of the analyzed traits.

Statistical Analyses

All analyses were performed using the statistical software SAS (SAS Institute, Inc., Cary, NC). Descriptive characteristics of quantitative traits were obtained using PROC MEANS. Allele frequencies were tabulated and compared by χ^2 analysis using PROC FREQ.

Genotype Analyses. Associations of the genotypes for the SNP in the CAST gene with CF, GFAT, LMA, LEANYL, FATYL, BONEYL, and HCW were evaluated using PROC MIXED, fitting the following model, which included the known SNP genotypes as fixed effects:

$$Y_{ijklm} = u + \text{Gen}_i + \text{Sex}_j + \text{Slg}_k + \beta_1\text{AN} + \beta_2\text{LI} \\ + \beta_3\text{CH} + \beta_4\text{SM} + \text{Sire}_l + e_{ijklm} \text{ (model 1)},$$

in which Y_{ijklm} is the trait measured in the animal m of the sex j and the slaughter group k having the CAST SNP genotype i and born from the sire l ; u is the overall mean for the trait; Gen_i is the effect of the genotype i for the SNP in the CAST gene (CC, CG, or GG); Sex_j is the fixed effect of the sex j (bull, heifer or steer); Slg_k is the fixed effect of the slaughter group k (61 levels); $\beta_1, \beta_2, \beta_3, \beta_4$ are the regression coefficients of the linear regressions on breed composition (proportion of Angus, Limousin, Charolais, and Simmental, respectively); Sire_l is the random effect of the sire l ; and e_{ijklm} is the residual random effect associated with animal m .

Slaughter groups were defined as animals from the same source (Commercial or Rockwood) with the same slaughter date. The slaughter date accounted for most of the feedlot-of-origin variation in the commercial cattle, because the majority of the slaughter dates (90%) had cattle from a single feedlot. All other slaughter dates (10%) had cattle from only 2 feedlots. If the latter slaughter dates were divided into 2 groups, the resulting number of animals within the corresponding slaughter group would become too small, so they were maintained as a single group.

Animals originated from 78 sires, and all sires were known and assumed unrelated. The average size of the paternal half-sib families was 8.1. The percentages of sires with less than 5, from 6 to 10, from 11 to 15,

and more than 15 offspring were 42.3, 23.1, 25.6, and 9.0%, respectively.

For analyzing the shear force measurements, CF was included as a linear covariate in the model to account for possible effects of i.m. fat on meat tenderness:

$$Y_{ijklm} = u + \text{Gen}_i + \text{Sex}_j + \text{Slg}_k + \beta_1 \text{AN} + \beta_2 \text{LI} \\ + \beta_3 \text{CH} + \beta_4 \text{SM} + \beta_5 \text{CF} + \text{Sire}_l + e_{ijklm} \text{ (model 2)},$$

in which Y_{ijklm} is the shear force measurement of the animal m of sex j and slaughter group k having the CAST SNP genotype i , and born from the sire l ; B_5 is the regression coefficient of the linear regression on CF; and all other terms in model 2 are as previously described for model 1.

Following Bertrand et al. (2001), phenotypic and genetic relationships between marbling and tenderness are not especially high but show a favorable direction, indicating that greater marbling is slightly associated with greater tenderness.

The repeated LM shear force measurements were analyzed individually within each postmortem aging period and as the average shear force measure across 5 of postmortem aging, using model 2. Because these analyses ignore the covariance structure due to the sequential nature of the data on each animal, a repeated measures analysis of tenderness was also carried out, which modeled the covariance structure and allowed comparison of genotypes and trends over aging times (Littell et al., 1998).

The repeated measures analysis was carried out by PROC MIXED of SAS, using the Random and Repeated statements. The same effects as in model 2 were fitted in addition to the fixed effects of aging time (postmortem days) and the genotype by aging time interaction and the random effect of animal, which was the subject on which repeated measures across aging times were taken. The covariance structure within animals was assumed as block diagonal with one 4×4 block (4 measures) per animal. A heterogeneous first order autoregressive covariance structure was used:

$$\begin{bmatrix} \sigma_1^2 & \sigma_1\sigma_2\rho & \sigma_1\sigma_3\rho^2 & \sigma_1\sigma_4\rho^3 \\ & \sigma_2^2 & \sigma_2\sigma_3\rho & \sigma_2\sigma_4\rho^2 \\ \text{sym.} & & \sigma_3^2 & \sigma_3\sigma_4\rho \\ & & & \sigma_4^2 \end{bmatrix},$$

in which σ_i^2 is the variance associated with the postmortem aging period i and ρ is the autoregressive parameter. The (co)variance components were estimated by the REML method.

A heterogeneous first order autoregressive covariance structure within animal was chosen based on preliminary analyses, in which different covariance structures were modeled and the best fitting model was selected using the Akaike information criterion (Akaike, 1973).

The numerator df to test the fixed effects was obtained using the option DDFM=BETWITHIN. This option divides the residual df into between-subject and within-subject portions, and a check is made whether a fixed effect changes within any subject. If so, the within-subject df are assigned to the effect; otherwise the between-subject df are assigned. Genotype least squares means for each postmortem aging period were obtained using the Estimate statement.

The effect of CAST SNP genotypes on MG was analyzed by χ^2 analysis (PROC FREQ) and as a linear trait, applying model 1. In this case, scores of 1, 2, and 3 were assigned to grades A, AA, and AAA, respectively.

Average allele substitution effects (Falconer and Mackay, 1996) were estimated using the same models used to estimate the genotype effects but replacing the classification effect of genotypes by a linear regression on the number of C alleles (0, 1, or 2).

The association of the number of C alleles with the percentage of unacceptably tough LM steaks (LM shear force > 5.7 kg) was also determined. Researchers at the USDA (Wheeler et al., 1997) showed that when peak shear force was above the threshold of 5.7 kg, 100% of consumers would rate a steak as unacceptably tough.

The percentage of animals from each genotype having a LM shear force above the threshold was calculated using both the observed shear force and the shear force adjusted for sex, slaughter group, breed, CF, and sire effects. In the latter case, the adjusted shear force was obtained using the residuals of model 2 used to analyze shear force within postmortem aging periods, as previously described, but without fitting the SNP genotype effect. These residuals were added to the expected value of an observation in a given postmortem aging period to produce the adjusted shear force. Expected values were the least squares means for the time (postmortem aging period) effect given by the repeated measures analysis. The association of the number of C alleles with the percentage of unacceptably tough steaks was evaluated by χ^2 analysis.

To keep reasonable probability values for Type I errors, 2 levels of tests were performed. For initial assessment of the results, an overall value of $P < 0.05$ (α) was used. For a more detailed review of the results, a modified Bonferroni correction was used (α/\sqrt{n} ; Mantel,

1980) to account for the number of tests. The value of n was determined using a trait-wise approach, grouping traits according to type (Ye, 2003). Traits were grouped into 2 groups as follows: carcass yield traits (LEANYL, FATYL, BONEYL, GFAT, LMA, and HCW) and meat quality traits (CF, MG, SFL2, SFL7, SFL14, SFL21, and SFS7). So n was equal to 6 and 7 for carcass yield and meat quality traits, respectively, with the corresponding modified Bonferroni significance levels of 0.020 and 0.019.

RESULTS

Genotypic and Allelic Frequencies

Results for genotypic and allelic frequencies are presented in Table 2. The overall observed genotypic fre-

Table 2. Allele frequencies of the SNP in the calpastatin (CAST) gene for each breed and in the whole beef population

Breed ²	No. of animals	CAST SNP allele ¹	
		C, %	G, %
Angus	12	62.5	37.5
Limousin	28	73.2	26.8
Charolais	8	68.8	31.2
Simmental	33	36.4	63.6
Other ³	547	63.9	36.1
Total	628	62.9	37.1

¹Allele frequency was significantly different between breeds ($P = 0.001$ by χ^2 test).

²Animals with breed composition $\geq 5/8$ for a given breed.

³Animals with breed composition $< 5/8$ for all breeds.

quencies (43.0%, 39.8%, and 17.2% for CC, CG, and GG, respectively) were not in agreement with Hardy-Weinberg equilibrium ($P = 0.001$). The allelic frequency was different ($P = 0.001$) between breeds; Simmental animals showed greater frequency of allele G (frequency = 0.64) than all other breeds (frequency < 0.38).

Genotype Effects

The genotypes for the CAST SNP did not significantly influence CF ($P = 0.75$), GFAT ($P = 0.53$), HCW ($P = 0.25$), and MG ($P = 0.40$ by χ^2 test, and $P = 0.70$ by F -test, treating MG as a linear trait).

Results of the analyses of LM tenderness within each postmortem aging period and using the average LM tenderness measure over the 4 measurements are presented in Table 3. Analyses of the SNP genotypes within each postmortem aging period showed a highly significant effect ($P = 0.007$) on SFL21 and a trend to affect SFL2 and SFL7 ($P \leq 0.07$). The CAST genotypes signifi-

cantly influenced ($P = 0.012$) the average LM tenderness (SFLavg) over the 21-d postmortem period; genotype CC had the most tender LM compared with genotypes CG and GG. The difference between the homozygous genotypes CC and GG was -0.38 ± 0.13 kg; the heterozygous genotype showed an intermediate tenderness.

Table 3 also presents the average allele substitution effects, which were significant ($P \leq 0.03$) for all measures of LM tenderness, except for SFL14, for which there was a trend ($P = 0.10$). The allele substitution effect ranged from -0.26 ± 0.11 kg to -0.13 ± 0.06 kg for tenderness evaluated at 2 and 21 d of aging, respectively.

Genotype effect on SFL21 and SFLavg and average allele substitution effect on SFL2 and SFLavg were significant after modified Bonferroni correction for trait-wise multiple tests ($P < 0.05$), providing strong evidence for the effect of the SNP on LM tenderness.

In line with the effect on LM tenderness, CAST SNP genotypes tended to affect SFS7 ($P = 0.14$), with increased tenderness in beef from genotype CC vs. CG and GG (-0.17 ± 0.09 kg and 0.10 ± 0.12 kg, respectively). However, the average allele substitution effect on SFS7 was small and did not differ from zero ($P = 0.22$; Table 3).

Results of the repeated measures analysis are presented in Table 4. A highly significant overall effect of genotypes across aging days ($P = 0.005$) was found; genotype CC had more tender LM (-0.32 ± 0.13 kg) than GG, and genotype CG had an intermediate tenderness. The genotype least squares means for each postmortem aging period are also shown in Table 4. Means were significantly different ($P < 0.05$) for LM tenderness at 2, 7, and 21 d postmortem; genotype CC showed increased tenderness. However, the difference between the homozygous genotypes CC and GG decreased from $-0.53 \pm$

Table 3. Calpastatin SNP effect on LM shear force (SFL) evaluated at 2, 7, 14, and 21 d of postmortem aging, on the average LM shear force across aging days (SFLavg), and on semitendinosus muscle shear force (SFS) evaluated at 7 d of postmortem aging

Item	Trait					
	SFL2	SFL7	SFL14	SFL21	SFLavg	SFS7
SNP genotype least squares mean \pm SE, kg						
CC	5.55 ^y	5.03 ^y	4.23 ^y	3.67 ^y	4.53 ^y	5.01 ^y
	± 0.20	± 0.15	± 0.13	± 0.11	± 0.12	± 0.11
CG	5.81 ^y	5.13 ^y	4.39 ^y	3.95 ^z	4.71 ^{yz}	5.18 ^y
	± 0.20	± 0.15	± 0.14	± 0.11	± 0.12	± 0.12
GG	6.06 ^y	5.41 ^y	4.44 ^y	3.87 ^{yz}	4.91 ^z	5.11 ^y
	± 0.25	± 0.18	± 0.16	± 0.14	± 0.15	± 0.14
P^1	0.06	0.07	0.22	0.007 [†]	0.012 [†]	0.14
Average allele substitution effect \pm SE, kg						
Effect	-0.26	-0.17	-0.12	-0.13	-0.19	-0.07
	± 0.11	± 0.08	± 0.07	± 0.06	± 0.06	± 0.06
P^1	0.018 [†]	0.032	0.10	0.025	0.003 [†]	0.22

^{y,z}Within a column, means without a common superscript letter differ ($P < 0.05$) for multiple comparisons by Bonferroni test.

[†]Significant effect ($P < 0.05$) after modified Bonferroni correction for trait-wise multiple tests.

¹Probability of the F -test for genotype or average allele substitution effect.

Table 4. Calpastatin SNP effect on LM shear force at 2, 7, 14, and 21 d of postmortem aging and on the overall LM shear force across aging days, and time (days of postmortem aging) effect on LM shear force, given by repeated measures analysis

Item	Time				Overall
	2	7	14	21	
SNP genotype least squares mean \pm SE, kg					
CC	5.41 ^y	4.90 ^y	4.35 ^y	3.79 ^y	4.61 ^y
	\pm 0.14	\pm 0.11	\pm 0.11	\pm 0.10	\pm 0.10
CG	5.71 ^{yz}	5.09 ^{yz}	4.48 ^y	4.03 ^z	4.83 ^z
	\pm 0.14	\pm 0.12	\pm 0.11	\pm 0.11	\pm 0.10
GG	5.94 ^z	5.27 ^z	4.52 ^y	4.00 ^{yz}	4.93 ^z
	\pm 0.19	\pm 0.15	\pm 0.15	\pm 0.13	\pm 0.12
<i>P</i> ¹ for overall effect on tenderness					0.005 [†]
Time effect least squares mean \pm SE, kg					
	5.69	5.09	4.45	3.94	
	\pm 0.11	\pm 0.10	\pm 0.10	\pm 0.09	
<i>P</i> ¹ for time effect on tenderness					0.000
Average allele substitution effect \pm SE, kg					
Effect	-0.27	-0.18	-0.10	-0.13	-0.15
	\pm 0.10	\pm 0.07	\pm 0.07	\pm 0.06	\pm 0.05
<i>P</i> ¹	0.006 [†]	0.013 [†]	0.17	0.027	0.002 [†]

^{y,z}Within a column, means without a common superscript letter differ ($P < 0.05$) for multiple comparisons by Bonferroni test.

[†]Significant effect ($P < 0.05$) after modified Bonferroni correction for trait-wise multiple tests.

¹Probability of the *F*-test for genotype, time, or average allele substitution effect.

0.20 kg to -0.21 ± 0.12 kg from 2 to 21 d of aging, respectively. These differences correspond to 25 and 20% of the phenotypic SD of the shear force measurements at 2 and 21 d postmortem, respectively.

There was a highly significant effect of aging time (postmortem aging periods) on LM tenderness ($P < 0.001$, Table 4). The least squares means for time effect showed a significant tenderization as the meat aged. There was a reduction of 0.60 ± 0.08 kg in shear force from 2 to 7 d postmortem, 0.64 ± 0.06 kg from 7 to 14 d, and 0.51 ± 0.05 kg from 14 to 21 d. The interaction genotype by aging time was not significant ($P = 0.64$, data not shown).

Table 4 also presents the average allele substitution effect estimated by repeated measures analysis, which was highly significant on the overall tenderness across the postmortem periods ($P = 0.002$) and significant on the measures of LM tenderness at different postmortem aging periods ($P < 0.03$), except for SFL14. The allele substitution effect for each postmortem aging period was estimated by fitting the regression on the number of C alleles nested within aging time. Similar to the previous analyses of individual postmortem aging periods, the allele substitution effect given by the repeated measures analysis ranged from -0.27 ± 0.10 kg to -0.13 ± 0.06 kg for tenderness evaluated at 2 and 21 d postmortem, respectively.

The genotype effect on the overall tenderness across postmortem aging periods and average allele substitution effect on SFL2 and SFL7, and on the overall tenderness across postmortem aging periods were significant after modified Bonferroni correction for trait-wise multiple tests ($P < 0.05$), providing again strong evidence for the effect of the CAST SNP on tenderness.

There was no significant effect of either breed ($P > 0.07$) or sex ($P > 0.20$) in all analyses of tenderness (both within postmortem aging periods and by repeated measures analysis). On the contrary, slaughter group showed a highly significant effect ($P < 0.001$) on tenderness in all analyses (data not shown).

The covariate CF had a highly significant linear effect on SFL7, SFL14, and SFL21 ($P < 0.007$), with an estimated change in shear force of -0.11 kg, -0.09 kg, and -0.07 kg per 1% increase in CF for SFL7, SFL14, and SFL21, respectively (data not shown).

Table 5 presents the association of the number of C alleles with the percentage of unacceptably tough LM steaks (LM shear force > 5.7 kg). The percentage of unacceptably tough steaks based on the observed shear force values was associated with the number of C alleles at 2 ($P = 0.001$) and 7 ($P = 0.05$) d postmortem. At 2 d postmortem, 31.5%, 32.8%, and 18.9% of the steaks from cattle carrying 0, 1, and 2 copies of C allele were unacceptably tough, respectively. The corresponding features for 7 d postmortem were 35.2, 26.0, and 23.3%, respectively.

The percentage of unacceptably tough steaks was also determined based on shear force values adjusted for sex, slaughter group, breed, CF, and sire effects. In this case, the percentage of unacceptably tough steaks was also associated with the number of C alleles at 2 ($P = 0.05$) and 7 ($P = 0.03$) d postmortem. At 2 d postmortem, 36.1, 36.8, and 27.4% of the steaks from cattle carrying 0, 1, and 2 copies of C allele were unacceptably tough, respectively. The corresponding features for 7 d postmortem were 34.3, 26.8, and 21.5%, respectively.

Table 6 shows that CAST SNP tended to influence LMA ($P = 0.12$) and LEANYL ($P = 0.12$) and signifi-

Table 5. Association of the number of calpastatin SNP C alleles with the percentage of unacceptably tough LM steaks at 2, 7, 14, and 21 d of postmortem aging

	Days of postmortem aging			
	2	7	14	21
Percentage calculated based on observed shear force ¹				
0	31.5	35.2	21.3	5.6
1	32.8	26.0	13.2	7.2
2	18.9	23.3	13.0	3.7
<i>P</i> ²	0.001	0.05	0.09	0.21
Percentage calculated based on adjusted shear force ¹				
0	36.1	34.3	9.3	0.9
1	36.8	26.8	9.2	4.0
2	27.4	21.5	8.2	1.9
<i>P</i> ²	0.05	0.033	0.90	0.15

¹The percentage of animals from each genotype having LM shear force above the threshold for unacceptably tough steak (shear force > 5.7 kg) was calculated either using the observed shear force or the shear force adjusted for sex, slaughter group, breed, chemical fat, and sire effects.

²Probability of the χ^2 test for the association of the number of C alleles with the percentage of unacceptably tough LM steaks.

cantly influenced FATYL ($P = 0.037$) and BONEYL ($P = 0.037$). The CC genotype tended to have smaller LMA ($-1.77 \pm 1.50 \text{ cm}^2$) and lower LEANYL ($-0.97 \pm 0.51\%$) with greater fat yield ($+1.44 \pm 0.56\%$) and lower bone yield ($-0.47 \pm 0.22\%$) than the GG genotype. The average allele substitution effects on FATYL and BONEYL were significant ($P < 0.02$), even after modified Bonferroni correction for trait-wise multiple tests ($P < 0.05$).

Sex had a highly significant effect ($P < 0.01$) on LMA and BONEYL; bulls had the largest LMA and the highest BONEYL. Slaughter group and breed had highly significant effect ($P < 0.001$) on LMA, LEANYL, FATYL, and BONEYL. Angus cattle had the smallest LMA and the highest FATYL, resulting in the lowest LEANYL. On the contrary, Limousin cattle had the largest LMA and the lowest FATYL, resulting in the highest LEANYL. Charolais and Simmental cattle showed intermediate performance (data not shown).

DISCUSSION

Alleles of the CAST SNP identified in this study were segregating in the beef population with overall greater frequency for C than G allele with the exception of Simmental animals that conversely showed greater incidence of G allele. This, however, may not represent the actual frequency of C in the Simmental breed because only a small sample of animals with breed composition $\geq 5/8$ Simmental ($n = 33$) was genotyped.

The allele C was associated with significant increase in LM tenderness across d of postmortem aging. The magnitude of the effect of the C allele in this study was similar to that reported for the GeneSTAR tender allele (Genetic Solutions Pty. Ltd., Albion, Australia, http://www.geneticsolutions.com.au/files/GeneSTAR/pdf/GeneNOTE_4.pdf), which was -0.37 kg on LM shear force at 7 d postmortem between animals carrying 2 or

Table 6. Calpastatin SNP effect on LM area (LMA), lean (LEANYL), fat (FATYL), and bone (BONEYL) yield

Item	Trait			
	LMA, cm^2	LEANYL, %	FATYL, %	BONEYL, %
SNP genotype least squares mean \pm SE				
CC	90.0 ^y	56.1 ^y	24.5 ^y	19.4 ^y
	± 1.45	± 0.51	± 0.56	± 0.20
CG	92.2 ^y	56.1 ^y	24.1 ^{yz}	19.8 ^z
	± 1.53	± 0.53	± 0.59	± 0.21
GG	91.8 ^y	57.1 ^y	23.1 ^z	19.9 ^z
	± 1.77	± 0.62	± 0.68	± 0.25
<i>P</i> ¹	0.12	0.12	0.037	0.037
Average allele substitution effect \pm SE				
Effect	-1.14	-0.41	0.67	-0.26
	± 0.17	± 0.25	± 0.27	± 0.11
<i>P</i> ¹	0.11	0.10	0.015 [†]	0.016 [†]

^{y,z}Within a column, means without a common superscript letter differ ($P < 0.05$) for multiple comparisons by Bonferroni test.

[†]Significant effect ($P < 0.05$) after modified Bonferroni correction for trait-wise multiple tests.

¹Probability of the *F*-test for genotype or average allele substitution effect.

no tender alleles. The polymorphism in the CAST gene used in the GeneSTAR test (Barendse, 2002) is, however, different from the one reported in the current study.

The difference in LM shear force at 7 d postmortem between homozygous genotypes CC and GG given by the repeated measures analysis (-0.37 ± 0.16 kg) was equivalent to 62% of the estimated difference between the shear force at 2 and 7 d postmortem (time effect, Table 4), which was a measure of the tenderization of the meat as it aged. The magnitude of the benefit of the favorable genotype can then be put in perspective, relative to the benefits of aging, which are known and are a costly means of increasing beef tenderness. Selection for the beneficial genotype could then either increase beef tenderness, at the same level of aging, or provide a cost savings to attain the same level of tenderness with less aging.

Assuming a heritability of 30% for tenderness (Koch et al., 1982) evaluated in any of the postmortem aging periods and using the phenotypic SD in Table 1, the average allele substitution effects given by the repeated measures analysis (which were similar to those from the analyses within postmortem aging periods) would correspond to 29, 22, 14, and 23% of the respective genetic SD for shear force at 2, 7, 14, and 21 d postmortem, respectively, which are large genetic effects for a single SNP.

The number of C alleles was significantly associated with the percentage of unacceptably tough LM steaks at 2 and 7 d postmortem. The percentage of unacceptably tough steaks at 7 d postmortem decreased by 24 and 35% for cattle carrying 1 and 2 copies, respectively, of C allele compared relatively with steaks from animals with 0 copies, regardless if the percentage was calculated based on the observed or adjusted shear force. For 2 d postmortem, the presence of 2 copies of the C allele was associated with lower percentage of unacceptably tough steaks based in both observed (41% lower) and adjusted shear force (25% lower) compared relatively with steaks from animals with 1 or 0 copies.

The need for 2 C alleles to reduce the percentage of unacceptably tough steaks at 2 d of aging was expected because the least squares mean for shear force at 2 d postmortem for the heterozygous genotype CG (Tables 3 and 4) was above the assumed toughness threshold (shear force >5.7 kg), making the effect of only 1 C allele masked in terms of reduced percentage of unacceptably tough steaks. At 14 and 21 d postmortem, there was not a clear association between the number of C alleles and the percentage of unacceptable tough steaks, likely because, at these 2 d of postmortem aging, the percentage of animals with steaks above the assumed toughness threshold was much smaller than at 2 and 7 d postmortem due to the tenderization of meat as it aged.

The magnitude of the association of number of C alleles with the percentage of unacceptably tough LM steaks at 7 d postmortem was smaller than that reported for the GeneSTAR tender allele ([\[eticsolutions.com.au/files/GeneSTAR/pdf/GeneNOTE_4.pdf\]\(http://www.geneticsolutions.com.au/files/GeneSTAR/pdf/GeneNOTE_4.pdf\)\), which was 33% and 62% for cattle carrying one and two copies of the tender allele relatively to animals with no copies, respectively.](http://www.gen-</p>
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Therefore, the CAST SNP C allele was associated with increased LM tenderness but tended to reduce LM area and lean yield and significantly increase fat yield. This evidence that the CAST SNP may also affect other important carcass traits, such as fat yield, warrants further investigation.

IMPLICATIONS

Alleles of the CAST single nucleotide polymorphism identified in this study were segregating in the beef population with an overall greater frequency for allele C than G. Allele C was associated with more tender LM across days of postmortem aging, but tended to reduce LM area and lean yield and increase fat yield. Importantly for the beef industry, the difference in the tenderness at 2 and 7 days of postmortem aging is expected to substantially reduce the percentage of steaks rated unacceptably tough by consumers based on an assumed threshold level. Thus, it is expected that single and double C cattle would produce more tender steaks and that this would result in significantly fewer unsatisfactory eating experiences.

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