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Effects of polymorphisms in the calpastatin and μ-calpain genes on meat tenderness in 3 French beef breeds[1]

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ABSTRACT: The objectives of the study were to evaluate allelic frequencies and to test the association of polymorphisms in the calpastatin (*CAST*) and μ-calpain (*CAPN1*) genes with meat tenderness in 3 French beef breeds. A total of 1,114 Charolais, 1,254 Limousin, and 981 Blonde d'Aquitaine purebred young bulls were genotyped for 3 SNP in the *CAST* gene and 4 SNP in the *CAPN1* gene. Two of these markers, 1 in each gene, can be found in Australian or American commercial genetic tests. Others have previously been reported in American studies or are newly evidenced SNP. The quantitative traits studied were Warner-Bratzler shear force and a tenderness score evaluated by trained sensory panels. All the SNP were informative in the 3 breeds. Associations of individual markers or haplotypes with traits were analyzed. The results differed in the 3 breeds. The G allele of a *CAST* marker (position 97574679 on Btau4.0) was found to exert a significant effect on the shear force (+0.18 phenotypic SD; RSD) and tenderness score (−0.22 RSD) in the Blonde d'Aquitaine breed. In the same breed, this marker was associated with another *CAST* SNP (position 97576054 on Btau4.0) such that the GA haplotype appeared to be associated with tougher meat. Two *CAPN1* markers (positions 45221250 and 45241089 on Btau4.0) had a significant effect on both traits in the Charolais breed (from |0.11| to |0.25| RSD). In the same breed, these markers were associated with another *CAPN1* SNP (position 45219395 on Btau4.0) such that the ACA and AGG haplotypes appeared to be associated with a tender meat and a tougher meat, respectively. Consequently, the present results indicate that the effects of the markers studied are breed-specific and cannot be extended to all *Bos taurus* breeds. Further studies are also required to identify other more appropriate markers for French beef breeds.

Key words: beef cattle, μ-calpain gene, calpastatin gene, meat quality, polymorphism

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INTRODUCTION

Meat tenderness is an important issue in beef cattle because it has a major impact on consumer satisfaction. However, beef meat quality is not routinely measured, so a classical selection based on records is not feasible. Under these conditions, a study of a molecular basis for variations in meat tenderness may provide a

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solution to improve tenderness by developing marker-assisted selection. Markers in the μ-calpain (***CAPN1***) and calpastatin (***CAST***) genes have been suggested as being associated with meat tenderness (Barendse, 2002; Page et al., 2002, 2004; Casas et al., 2005, 2006; White et al., 2005; Schenkel et al., 2006). The *CAPN1* gene, mapped on chromosome 29 (Smith et al., 2000), encodes the protease μ-calpain, which degrades myofibrillar proteins postmortem, and the *CAST* gene, mapped on chromosome 7 (Bishop et al., 1993), encodes its inhibitor (Koohmaraie, 1996). Moreover, genetic tests for meat tenderness which utilize genetic polymorphisms in the *CAST* and *CAPN1* genes have been marketed as Igenity TenderGENE (Merial Ltd., Atlanta, GA) and GeneSTAR Elite Tender (Genetic Solutions Pty. Ltd., Albion, Australia). The effects of these genetic tests were validated by the National Beef Cattle Evaluation Consortium in the United States (Van Eenennaam et al., 2007). The effects of *CAST* and *CAPN1* gene markers were assessed in *Bos taurus*, *Bos indicus*, and *B. taurus* × *B. indicus* populations but have not so far been investigated in French purebred populations. The objectives of this study were thus to take advantage of the highly informative French progeny testing system for AI sires to evaluate 1) the frequencies of genotypes for markers in the *CAST* and *CAPN1* genes in the Charolais, Limousin, and Blonde d'Aquitaine breeds, and 2) the effects of these polymorphisms on meat tenderness in the 3 breeds.

MATERIALS AND METHODS

The animals used during this study were slaughtered in accredited slaughterhouses according to the rules on animal protection defined by French law (Code Rural, articles R214–64 to R214–71, http://www.legifrance.gouv.fr).

The Qualvigène program, described in detail elsewhere (Allais et al., 2010), was a collaborative research program involving AI companies, INRA (the French National Institute for Agricultural Research) and the Institut de l'Elevage (Breeding Institute) in France. The program was initiated to study the genetic determinism of beef and meat quality traits (Malafosse et al., 2007). The study formed an integral part of this Qualvigène program.

Animals

The Qualvigène program was based on 3 successive year progeny tests. The population used in this study has been described in detail elsewhere (Allais et al., 2010). Briefly, purebred young bulls, the progeny of 48 Charolais, 36 Limousin, and 30 Blonde d'Aquitaine sires, were randomly procreated in a large number of herds from mostly unrelated dams. In each breed and each year, calves were born within a restricted period of 17 wk. After weaning in the farm of origin, bull calves entered the feedlots at 40, 37, or 24 wk of age on average (±3 wk) for the Charolais, Limousin, and Blonde d'Aquitaine breeds, respectively. The Charolais bull calves were fattened at 2 locations and fed ad libitum with whole plant corn silage. The Limousin and Blonde d'Aquitaine bull calves were fattened in a single feedlot and fed ad libitum with a corn-based finishing diet. They were humanely slaughtered in commercial slaughterhouses (a single slaughterhouse for each feedlot) when they reached 730 kg (±15 kg) BW on average for the Charolais progeny and 479 d (±3 d) or 417 d (±4 d) on average for the Limousin or Blonde d'Aquitaine progeny, respectively. For each slaughter batch (13 to 26 young bulls), the reduction in temperature during chilling was monitored using sensors inserted between the 10th and 11th ribs of 2 or 3 half-carcasses. A total of 1,114 Charolais, 1,254 Limousin, and 981 Blonde d'Aquitaine purebred young bulls were thus used in this study.

Traits Evaluated

The traits analyzed were meat tenderness attributes: Warner-Bratzler shear force and tenderness score (Table 1). It had previously been agreed with the AI companies that the project would not constitute a breed comparison study. The longissimus thoracis muscle (**LT**) was excised 24 h postmortem from the 8th and 9th ribs of the right half carcass and sliced into 2 steaks that were vacuum-packaged and kept at 4°C for 14 d for aging, before being frozen. Before each experimental session, the steaks were placed at 4°C for 24 h to thaw and were then cooked over an electric grill to an internal temperature of 55°C (rare cooking), using standardized cooking equipment, cooking temperatures, and cooking times. Cooked steaks from the 8th rib was cooled to room temperature before 10 parallelepiped core samples were cut up with the fibers oriented parallel to the long axis. The Warner-Bratzler shear force (N/cm^2) was averaged on the 10 core measures. Cooked steaks from the 9th rib were served immediately to the panelists. Tenderness was evaluated by 3 different test panels, 1 for each breed and each composed of 12 trained panelists, most of whom were involved for the 3 yr of the study. The Blonde d'Aquitaine test panel was asked to evaluate 12 samples during each session, and the Charolais and Limousin test panels were asked to evaluate 15 samples. The panelists scored tenderness on nonstructured 100-point scales from 1 (extremely tough) to 100 (extremely tender). The scores were averaged over the panelists for each animal.

Phenotypic correlations between the 2 traits were −0.43, −0.34, and −0.36, whereas the genetic correlations were −0.91, −0.91, and −0.86 in the Charolais, Limousin, and Blonde d'Aquitaine breeds, respectively (unpublished results).

SNP Genotyping

Three and 4 SNP in the bovine *CAST* and *CAPN1* genes, respectively, were genotyped. Detailed informa-

Table 1. Overall means, number of observations, and phenotypic SD (RSD[1]) for shear force and the tenderness score in the 3 breeds

Trait	Overall mean[2]	Charolais		Limousin		Blonde d'Aquitaine	
		No.	RSD	No.	RSD	No.	RSD
Shear force, newtons/cm^2	39.9	1,114	7.32	1,252	7.47	977	10.41
Tenderness score	60.8/100	1,113	7.87	1,241	7.25	970	10.72

[1]RSD: root mean square error of the basic model that includes only the fixed effect of the contemporary groups.
[2]It was agreed with the AI companies that this project would not constitute a breed comparison study.

tion on the locations and positions of the markers is given in Table 2. The *CAST-2* and *CAPN1–2* markers were included in the Australian GeneSTAR Tenderness commercial test, and the *CAPN1–2* marker was included in the American Igenity Tenderness commercial test.

Deoxyribonucleic acid was extracted from blood samples (1 mL) from all calves, from blood samples (1 mL) from 78% of the dams and from blood (1 mL) or semen (0.22 to 0.25 mL) samples from all the sires. Genotyping was performed using TaqMan SNP genotyping assays designed by Applied Biosystems (Courtaboeuf, France) with an ABI 7900HT Real-Time PCR System (Applied Biosystems). The primers used are shown in Table 3. We investigated a total of 6 and 8 SNP in the *CAST* and *CAPN1* genes, respectively, but several of them were discarded because they were not informative (they were not polymorphic in our breeds or were in complete disequilibrium with another of the markers further studied; data not shown). It should be noted that one of the *CAST* SNP located in exon 30 (A/T position 97574736 on Btau 4.0) described by Barendse (2002) did not display any polymorphism in any of the 3 breeds examined.

Statistical Analysis

The models were evaluated using the Mixed procedure (SAS Inst. Inc., Cary, NC). All analyses were performed separately for each breed. To study individual associations between each marker and shear force or the tenderness score at the population level, a mixed model of ANOVA and a mixed model of regression were employed. The mixed model of ANOVA was

$$Y_{ijkl} = \mu + C_i + G_j + S_k + e_{ijkl},$$

where Y_{ijkl} = phenotypic observations; μ = overall mean; C_i = fixed effect of contemporary group (i.e., cattle from the same fattening lot and slaughtered on the same day for shear force), or the date of the tasting panel for the tenderness score; G_j = fixed effect of genotypes; S_k = random effect of sire; and e_{ijkl} = random error. This first model was used to test for the dominance effect, estimated by the contrast between the effect of the heterozygous genotype and the average effect of the 2 homozygous genotypes. This effect and the associated probability values were obtained by means of the estimate option of the SAS Mixed procedure. When no dominance effect was observed, the more powerful regression model was performed to estimate the additive effect. This model was a regression on the number of copies of 1 of the 2 alleles present in the genotype, assuming additive effects for this marker. For example, for an A/G polymorphism, genotype AA was coded 0, AG 1, and GG 2. The other effects were the same as in the first model. The probability values were not corrected for multiple testing.

To study the effect of haplotypes segregating in the populations, a mixed model of multiple regression was used:

$$Y_{ijkl} = \mu + C_i + \sum_{1}^{t} \beta_j H_j + S_k + e_{ijkl},$$

Table 2. Information on the SNP genotyped[1]

Gene	BTA	SNP name	Other published name	Location	Position on Btau 4.0	SNP	AA substitution
CAST	7	*CAST-1*		Intron 8	97531815	A/G	
		CAST-2	CAST-T1	Exon 30 (3′UTR)	97574679	A/G	
		CAST-3		Exon 30 (3′UTR)	97576054	A/G	
CAPN1	29	*CAPN1–1*		Exon 6	45219395	A/G	
		CAPN1–2	CAPN316	Exon 9	45221250	C/G	Gly/Ala
		CAPN1–3	CAPN530	Exon 14	45237834	A/G	Ile/Val
		CAPN1–4		Intron 19	45241089	A/G	

[1]*CAST-1* and *CAST-3* were newly evidenced in resequencing regions of the bovine calpastatin (*CAST*) gene (unpublished data). *CAST-2* was reported by Barendse (2002). All μ-calpain (*CAPN1*) markers were reported by Page et al. (2002). UTR = untranslated region.

Table 3. Primers used

SNP name[1]	Forward primer	Reverse primer	Probe 1	Probe 2
CAST-1	CAGGCCAGATTTTAACCATTTTGATAGC	CTGACTGGAAAACAGAGCAGATATGT	ATGTCAAAGTAAAGGATGTG	TCAAAGTGAAGGATGTG
CAST-2	CTCACGTGTTCTTCAGTGTTCTG	CAACCCAAAGAAACATCAAACACAGT	CCTTTCCTCTTAGACTTG	CTTTCCTCTTGGACTTG
CAST-3	TGATGAACTGAGTAAGCTACAGGATCT	AAAATGTTATTTACCTCTAAGCAAGTATTTGCAATT	CCCATCTAATTCACAGTAAA	CCATCTAATTCGCAGTAAA
CAPN1–1	AGCTACGAGGCCCTCTCA	TTGCGCAGCTCGTACCA	CCCTCAGATGTGCTGC	CCTCAGACGTGCTGC
CAPN1–2	GCAGTGCCGTTTTCCTACAG	AGCTGCTCCCGCATGTAAG	CCACGGCGTTCCA	CCACGCCGTTCCA
CAPN1–3	TTGACTGGCCCCTCTCTCT	GGCAGGGCACGTACCT	ATGACCAGGTCCAGGC	ATGACCAGATCCAGGC
CAPN1–4	CACAGTCCCACTTCAGGATGAG	CCAGGAGCTGGCCATCAG	TTTCCGAACAGATGAA	CCGAGCAGATGAA

[1]*CAST* = calpastatin; *CAPN1* = μ-calpain.

where μ, C_i, S_k, and e_{ijkl} were the same effects as in the marker models and H_j was the number of copies of each haplotype j (H_j = 0, 1, or 2), with t the number of haplotypes segregating in the population and β the regression coefficient. The contrast option of the SAS Mixed procedure was used to test the overall effect of haplotypes (df = t − 1), and the estimate option of the Mixed procedure was used to test the differences between 2 haplotype effects (df = 1). The regression coefficient of each haplotype was expressed as a deviation from the average of all regression coefficients, which was set to zero. Haplotype reconstruction was implemented using a program developed at INRA (Druet et al., 2008). Briefly, during a first step, alleles from homozygous SNP were assigned to both haplotypes of a given animal. As a second step, in offspring for which allele origin (maternal and paternal) could be determined unambiguously (conditionally on sire genotype), alleles were assigned accordingly to the corresponding haplotype. Third, the most likely sire haplotypes were then constructed sequentially. And finally, unassigned markers in offspring were determined with the help of neighboring markers that had already been assigned and using parental haplotypes. For all analyses, all available phenotypes were used, including those of animals with missing genotypes (not genotyped or failed genotyping), by adding a factor to the model (yes or no) depending on whether the animal was genotyped or not. Markers and haplotypes were nested within "yes." "No" included all animals with missing genotypes. The inclusion of missing genotypes prevented a loss of data and was important to ensuring a fair estimation of the fixed effect of contemporary groups.

The effects of haplotypes segregating within heterozygous sires were estimated using the following model: $Y_{ijkl} = \mu + C_i + S_k + H(S)_{kj} + e_{ijkl}$, where Y_{ijkl}, μ, C_i, S_k, and e_{ijkl} had the same definition as in the previous models. The $H(S)_{kj}$ effect referred to that of the transmitted haplotype H_j within sire S_k. The within-sire haplotype effect was estimated by contrasting the performances of progeny that unequivocally received haplotype H_j from the sire, and the performances of progeny that did not receive this haplotype.

RESULTS

Frequencies of Genotypes, Alleles, and Haplotypes

Table 4 shows the genotypic and allelic frequencies for the 7 markers studied in young bulls from 3 breeds. Allelic frequencies were relatively similar across the 3 breeds for each of the 3 SNP of the *CAST* gene. The G allele frequencies were about 80% for *CAST-1*, 20% for *CAST-2*, and 30% for *CAST-3*. Concerning *CAST-1*, the frequency of the G allele in the Charolais breed was less than in the other 2 breeds. Regarding *CAPN1–1*, there were more AA animals than GG animals in the Limousin breed, unlike the other 2 breeds, so the fre-

Table 4. Numbers of young bull genotypes and allelic frequencies for the 7 markers and the 3 breeds

			Charolais		Limousin		Blonde d'Aquitaine	
Marker[1]	Genotype	Allele	n	Frequency	n	Frequency	n	Frequency
CAST-1	AA		61		24		15	
	AG		413		303		235	
	GG		600		910		718	
		G		0.75		0.86		0.86
CAST-2	AA		738		838		568	
	AG		321		384		358	
	GG		35		23		45	
		G		0.18		0.17		0.23
CAST-3	AA		453		606		566	
	AG		508		522		355	
	GG		126		110		47	
		G		0.35		0.30		0.23
CAPN1–1	AA		137		337		40	
	AG		506		613		298	
	GG		451		255		620	
		G		0.64		0.47		0.80
CAPN1–2	CC		4		87		3	
	CG		178		492		77	
	GG		902		634		887	
		G		0.91		0.73		0.96
CAPN1–3	AA		58		151		129	
	AG		400		591		439	
	GG		635		491		396	
		G		0.76		0.64		0.64
CAPN1–4	AA		319		222		143	
	AG		567		645		432	
	GG		203		368		389	
		G		0.45		0.56		0.63

[1]*CAST* = calpastatin; *CAPN1* = μ-calpain.

quency of the G allele (47%) was less than in Charolais (64%) and Blonde d'Aquitaine (80%) animals. For *CAPN1–2*, the frequency of the G allele was greater in the Charolais (91%) and Blonde d'Aquitaine breeds (96%) compared with the Limousin breed (73%). The number of CC genotypes in these 2 breeds was less than 1%. The frequency of the G allele for *CAPN1–3* was about 70% in the 3 breeds and that of the G allele for *CAPN1–4* ranged from 45% in Charolais to 63% in Blonde d'Aquitaine. Among the 21 situations, we found only 2 cases where genotypic frequencies deviated from the expected Hardy-Weinberg proportions: the *CAST-2* [$\chi^2 = 7.86$, $P = 0.005$) and *CAPN1–4* ($\chi^2 = 4.35$, $P = 0.04$)] markers in the Limousin breed.

Table 5 shows the haplotypic frequencies of the *CAST* and *CAPN1* genes in the 3 breeds. Haplotypic diversity was relatively similar across the 3 breeds for the 2 genes. Concerning the *CAST* gene, 6 of the 8 possible haplotypes were found. Five of them were observed in all 3 breeds, and 1 (GGA) was only present in the Blonde d'Aquitaine breed. The most frequent haplotype was GAA in the 3 breeds (more than 60%) and the least frequent was GAG in the Charolais and Limousin breeds. In the Blonde d'Aquitaine breed, the frequencies of AGG and GAG haplotypes were low (about 1%). Concerning the *CAPN1* gene, we found 9 of the 16 possible haplotypes in the Charolais, Limousin, and Blonde d'Aquitaine breeds. The most frequent haplotype in the Limousin breed was the ACGA haplotype (20.1%), whereas it was one of the least frequent in the Blonde d'Aquitaine breed (3.0%). The frequency of this haplotype in the Charolais breed was 5.7%. The most frequent haplotypes were GGGA in the Charolais breed (30.2%) and GGAG in the Blonde d'Aquitaine breed (30.4%). Animals carrying haplotypes with a very low frequency (<1%) were not used during the analyses of haplotype effects.

Marker Associations

Among the 21 ANOVA analyses, and except for the *CAPN1–3* marker in the Charolais breed, we determined no significant dominant effect. Consequently, we decided to calculate the marker additive effects using the regression model (Tables 6 and 7). To compare the effects on both traits, the tables also include the ratio of each additive effect to the residual SD (**RSD**) of the base model that included only the contemporary group effects. In the Charolais breed there was a significant effect of the *CAST-3* marker on shear force (+0.12 RSD, $P = 0.01$) but not on the tenderness score. There were significant effects of the *CAPN1–2* and *CAPN1–4*

Table 5. Numbers and frequencies of young bull haplotypes in the 3 breeds[1]

	Charolais		Limousin		Blonde d'Aquitaine	
Haplotype	n	Frequency	n	Frequency	n	Frequency
CAST						
GAA	1,372	0.65	1,698	0.71	1,214	0.63
GGA	0	0.00	0	0.00	260	0.14
AAG	307	0.15	209	0.09	242	0.13
GAG	45	0.02	80	0.03	22	0.01
AGG	212	0.10	129	0.05	20	0.01
GGG	162	0.08	288	0.12	160	0.08
Total	2,098	1.00	2,404	1.00	1,918	1.00
CAPN1						
ACAG	3	0.002	66	0.03	6	0.004
ACGA	109	0.06	432	0.20	51	0.03
ACGG	49	0.03	86	0.04	9	0.01
AGAG	114	0.06	284	0.13	86	0.05
AGGA	387	0.20	259	0.12	98	0.06
AGGG	3	0.002	26	0.01	66	0.04
GGAG	320	0.17	425	0.20	518	0.30
GGGA	580	0.30	252	0.12	486	0.29
GGGG	355	0.18	324	0.15	384	0.23
Total	1,920	1.00	2,154	1.00	1,704	1.00

[1]*CAST* = calpastatin; *CAPN1* = μ-calpain.

markers on both traits in the Charolais breed: the G allele was associated with tougher meat for the 2 markers. The effect of the G allele of *CAPN1–2* was +0.22 RSD on shear force ($P = 0.01$) and −0.25 RSD on the tenderness score ($P = 0.002$), and the effect of the G allele of *CAPN1–4* was +0.11 RSD ($P = 0.03$) on shear force and −0.11 RSD on the tenderness score ($P = 0.03$). In the Limousin breed, we demonstrated a significant effect for the G allele of the *CAPN1–2* marker on shear force (+0.12 RSD, $P = 0.02$) but not on the tenderness score, and a significant effect of the G allele of the *CAST-1* marker on the tenderness score (−0.17 RSD, $P = 0.01$) but not on shear force. In the Blonde d'Aquitaine breed, the G allele of the *CAST-2* marker was associated with tougher meat when studying the 2 traits (+0.18 RSD of shear force, $P = 0.002$, and −0.22 RSD of meat tenderness, $P = 0.0002$).

Haplotype Associations

The results of haplotype analyses are reported in Tables 8 and 9 for the Charolais breed, Table 10 for the Limousin breed, and Tables 11 and 12 for the Blonde d'Aquitaine breed. The individual effects of each haplotype are shown in Table 8 for the Charolais breed (ordered by their increasing effect on shear force). In this breed, the 5 *CAST* haplotypes exerted a significant effect on the tenderness score ($P = 0.01$), but not on shear force ($P = 0.14$). The AGG haplotype was associated with more tender meat according to the panelists (+0.22 RSD). We found a highly significant effect for the 7 *CAPN1* haplotypes on shear force ($P = 0.002$) and on the tenderness score ($P = 0.001$) in the Charolais breed. The ACGA haplotype appeared to be associated with more tender meat, in light of the shear force

Table 6. In the 3 breeds studied, additive effects of the G allele with SE, divided by the phenotypic SD (RSD[1]) for shear force, with the corresponding *P*-values

	Charolais			Limousin			Blonde d'Aquitaine		
Marker[2]	a ± SE	a/RSD	*P*-value	a ± SE	a/RSD	*P*-value	a ± SE	a/RSD	*P*-value
CAST-1	−0.56 ± 0.42	−0.08	0.18	0.62 ± 0.46	0.08	0.18	0.64 ± 0.82	0.06	0.43
CAST-2	0.78 ± 0.45	0.11	0.08	−0.24 ± 0.46	−0.03	0.59	1.86 ± 0.61	0.18	0.002
CAST-3	0.91 ± 0.37	0.12	0.01	−0.03 ± 0.36	−0.004	0.93	−0.61 ± 0.64	−0.06	0.34
CAPN1–1	−0.24 ± 0.36	−0.03	0.51	−0.04 ± 0.32	−0.005	0.91	−0.87 ± 0.67	−0.08	0.20
CAPN1–2	1.60 ± 0.61	0.22	0.01	0.90 ± 0.37	0.12	0.02	−0.49 ± 1.24	−0.05	0.69
CAPN1–3	−0.51 ± 0.41	−0.07	0.21	−0.40 ± 0.34	−0.05	0.24	0.80 ± 0.54	0.08	0.14
CAPN1–4	0.78 ± 0.35	0.11	0.03	0.11 ± 0.33	0.01	0.74	0.32 ± 0.52	0.03	0.54

[1]RSD: root mean square error of the basic model that includes only the fixed effect of the contemporary groups.
[2]*CAST* = calpastatin; *CAPN1* = μ-calpain.

Table 7. In the 3 breeds studied, additive effects of the G allele with SE, divided by the phenotypic SD (RSD[1]) for the tenderness score, with the corresponding *P*-values

	Charolais			Limousin			Blonde d'Aquitaine		
Marker[2]	a ± SE	a/RSD	*P*-value	a ± SE	a/RSD	*P*-value	a ± SE	a/RSD	*P*-value
CAST-1	0.12 ± 0.45	0.02	0.79	−1.20 ± 0.45	−0.17	0.01	−1.05 ± 0.86	−0.10	0.22
CAST-2	0.45 ± 0.48	0.06	0.36	−0.27 ± 0.45	−0.04	0.54	−2.40 ± 0.64	−0.22	0.0002
CAST-3	−0.53 ± 0.40	−0.07	0.18	0.25 ± 0.35	0.03	0.48	0.57 ± 0.67	0.05	0.40
CAPN1–1	0.29 ± 0.39	0.04	0.46	0.36 ± 0.32	0.05	0.26	1.29 ± 0.70	0.12	0.07
CAPN1–2	−1.99 ± 0.65	−0.25	0.002	0.08 ± 0.37	0.01	0.83	0.67 ± 1.32	0.06	0.61
CAPN1–3	0.19 ± 0.43	0.02	0.66	0.36 ± 0.33	0.05	0.28	−0.04 ± 0.57	−0.003	0.95
CAPN1–4	−0.83 ± 0.37	−0.11	0.03	0.14 ± 0.32	0.02	0.67	−0.43 ± 0.55	−0.04	0.44

[1]RSD: root mean square error of the basic model that includes only the fixed effect of the contemporary groups.
[2]*CAST* = calpastatin; *CAPN1* = μ-calpain.

and tenderness score results (−0.25 and +0.33 RSD, respectively). The AGAG haplotype was associated with tougher meat (+0.20 RSD on shear force and −0.19 RSD on the tenderness score). Among the 4 *CAPN1* SNP, discarding *CAPN1–3* enabled the pooling of 2 intermediate haplotypes and maintenance of a marked contrast between the 2 extreme haplotypes (Table 9), and the same overall effect of haplotypes on shear force ($P = 0.002$) and the tenderness score ($P = 0.001$). The ACA haplotype was associated with decreased shear force (−0.24 RSD) and an increased tenderness score (+0.30 RSD) and the AGG haplotype was associated with an increased shear force (+0.22 RSD) and a decreased tenderness score (−0.22 RSD).

The individual effects of each haplotype are reported in Table 10 for the Limousin breed (ordered by their increasing effect on shear force). In this breed, the 5 haplotypes of the *CAST* gene exerted a significant effect on the tenderness score ($P = 0.01$) but not on shear force ($P = 0.29$). There was a significant difference between the effects of the AGG haplotype (+0.16 RSD) and the GGG haplotype (−0.21 RSD), according to the panelists. There was no effect of the 9 *CAPN1* gene haplotypes on the 2 traits in the Limousin breed ($P = 0.07$ for shear force and $P = 0.62$ for the tenderness score).

The individual effects of each haplotype are shown in Table 11 for the Blonde d'Aquitaine breed (ordered by their increasing effects on shear force). In this breed, the 7 *CAPN1* haplotypes only exerted a significant effect on shear force ($P = 0.03$) and not on the tenderness score ($P = 0.27$), whereas there was a highly significant effect of the 6 *CAST* haplotypes on shear force ($P = 0.0004$) and the tenderness score ($P = 0.001$, Table 11). More precisely, the GGA haplotype increased shear force (+0.30 RSD) and decreased the tenderness score (−0.26 RSD). Among the 3 *CAST* SNP, discarding *CAST-1* allowed the remaining 4 haplotypes to exert a highly significant effect on shear force ($P < 0.0001$) and on the tenderness score ($P = 0.0002$). The GA

Table 8. Additive effects of each haplotype of the 3 calpastatin (*CAST*) gene markers and each haplotype of the 4 μ-calpain (*CAPN1*) gene markers with SE, divided by the phenotypic SD (RSD[1]) for shear force and the tenderness score, with the corresponding *P*-values, for the Charolais breed

	Shear force				Tenderness score			
Haplotype	a ± SE	a/RSD		*P*-value	a ± SE	a/RSD		*P*-value
CAST								
GAA	−0.87 ± 0.36	−0.12	a	0.02	0.37 ± 0.39	0.05	a	0.33
AAG	−0.24 ± 0.48	−0.03	ab	0.61	−0.76 ± 0.51	−0.10	b	0.14
AGG	−0.10 ± 0.53	−0.01	ab	0.84	1.71 ± 0.56	0.22	c	0.002
GGG	0.51 ± 0.58	0.07	b	0.38	−0.87 ± 0.62	−0.11	ab	0.16
GAG	0.71 ± 0.98	0.10	ab	0.47	−0.46 ± 1.06	−0.06	abc	0.66
CAPN1								
ACGA	−1.85 ± 0.68	−0.25	a	0.01	2.60 ± 0.73	0.33	a	0.0004
GGGA	−0.62 ± 0.37	−0.08	ab	0.10	0.55 ± 0.40	0.07	bc	0.17
ACGG	−0.51 ± 0.94	−0.07	abc	0.58	0.03 ± 0.99	0.004	abcd	0.97
GGAG	−0.04 ± 0.43	−0.01	bc	0.92	0.08 ± 0.46	0.01	cd	0.87
AGGA	0.78 ± 0.42	0.11	c	0.07	−0.87 ± 0.46	−0.11	d	0.06
GGGG	0.79 ± 0.43	0.11	c	0.07	−0.91 ± 0.46	−0.12	d	0.05
AGAG	1.46 ± 0.69	0.20	c	0.03	−1.49 ± 0.73	−0.19	d	0.04

[a–d]For each trait, haplotypes without a common letter differ ($P < 0.05$).
[1]RSD: root mean square error of the basic model that includes only the fixed effect of the contemporary groups.

Table 9. Additive effects of each haplotype of markers 1, 2, and 4 of the μ-calpain (*CAPN1*) gene in the Charolais breed with SE, divided by the phenotypic SD (RSD[1]) for shear force and the tenderness score, with the corresponding *P*-values

		Shear force				Tenderness score			
Haplotype	n	a ± SE	a/RSD		*P*-value	a ± SE	a/RSD		*P*-value
ACA	110	−1.79 ± 0.67	−0.24	a	0.01	2.39 ± 0.71	0.30	a	0.001
GGA	587	−0.58 ± 0.38	−0.08	a	0.13	0.41 ± 0.41	0.05	b	0.32
ACG	52	−0.47 ± 0.89	−0.06	ab	0.60	0.45 ± 0.94	0.06	abc	0.63
GGG	696	0.47 ± 0.35	0.06	b	0.18	−0.50 ± 0.37	−0.06	b	0.18
AGA	393	0.78 ± 0.43	0.11	b	0.07	−1.00 ± 0.46	−0.13	c	0.03
AGG	120	1.60 ± 0.66	0.22	b	0.02	−1.74 ± 0.70	−0.22	c	0.01

[a–c]For each trait, haplotypes without a common letter differ ($P < 0.05$).
[1]RSD: root mean square error of the basic model that includes only the fixed effect of the contemporary groups.

haplotype was associated with an increased shear force (+0.27 RSD) and a decreased tenderness score (−0.22 RSD; Table 12).

The Effects of Haplotypes Within Heterozygous Sire Families

Figure 1 illustrates the within-sire analysis and shows the effect of the GA *CAST* haplotype within Blonde d'Aquitaine sire families. Nine Blonde d'Aquitaine sires carried 1 copy of the GA haplotype. In most of these 9 families, the meat of progeny that received the GA haplotype from their sire had a greater shear force ($P = 0.05$ for the 9-contrast Fisher test) than that of progeny receiving another haplotype from the sire. However, the substitution effect of this haplotype was close to zero for tenderness score. The small number of young bulls per family limited any accurate estimates of the within-sire substitution effect, but these within family results seemed to confirm the results at the population level: the GA haplotype was associated with meat toughness in the Blonde d'Aquitaine breed. This analysis was not performed in the Charolais breed on the ACA *CAPN1* haplotype because of the small number of heterozygous ACA sires available.

DISCUSSION

Allelic and Genotypic Frequencies

The primary objective of this study was to evaluate the allelic frequencies of markers genotyped in the *CAST* and *CAPN1* genes in the Charolais, Limousin, and Blonde d'Aquitaine breeds. We focused in particular on the informativity of the markers included in commercial tests. In the *CAST* gene, the polymorphism of the *CAST-2* marker has been studied elsewhere in *B. taurus*, *B. indicus*, and *B. taurus* × *B. indicus* breeds

Table 10. Additive effects of each haplotype of the 3 calpastatin (*CAST*) gene markers and each haplotype of the 4 μ-calpain (*CAPN1*) gene markers with SE, divided by the phenotypic SD (RSD[1]) for shear force and the tenderness score, with the corresponding *P*-values, for the Limousin breed

	Shear force				Tenderness score			
Haplotype	a ± SE	a/RSD		*P*-value	a ± SE	a/RSD		*P*-value
CAST								
AGG	−1.07 ± 0.62	−0.14	a	0.08	1.17 ± 0.60	0.16	a	0.05
AAG	−0.36 ± 0.52	−0.05	ab	0.49	0.14 ± 0.50	0.02	ab	0.79
GAA	−0.10 ± 0.31	−0.01	ab	0.74	−0.42 ± 0.30	−0.06	b	0.16
GGG	0.11 ± 0.47	0.01	ab	0.82	−1.54 ± 0.46	−0.21	c	0.001
GAG	1.42 ± 0.76	0.19	b	0.06	0.64 ± 0.73	0.09	ab	0.38
CAPN1								
ACGA	−1.18 ± 0.44	−0.16	a	0.01	0.04 ± 0.44	0.01	a	0.92
ACGG	−0.66 ± 0.79	−0.09	abc	0.40	−0.09 ± 0.79	−0.01	a	0.91
GGGG	−0.36 ± 0.46	−0.05	ab	0.44	0.54 ± 0.46	0.07	a	0.23
GGGA	−0.10 ± 0.53	−0.01	abc	0.84	0.03 ± 0.53	0.01	a	0.95
ACAG	−0.06 ± 0.92	−0.01	abc	0.95	−0.98 ± 0.90	−0.13	a	0.28
GGAG	0.05 ± 0.43	0.01	bc	0.91	−0.17 ± 0.42	−0.02	a	0.69
AGAG	0.28 ± 0.49	0.04	bc	0.56	−0.41 ± 0.48	−0.06	a	0.39
AGGG	0.93 ± 1.44	0.12	abc	0.52	1.65 ± 1.38	0.23	a	0.23
AGGA	1.10 ± 0.51	0.15	c	0.03	−0.62 ± 0.50	−0.09	a	0.22

[a–c]For each trait, haplotypes without a common letter differ ($P < 0.05$).
[1]RSD: root mean square error of the basic model that includes only the fixed effect of the contemporary groups.

Table 11. Additive effects of each haplotype of the 3 calpastatin (*CAST*) gene markers and each haplotype of the 4 μ-calpain (*CAPN1*) gene markers with SE, divided by the phenotypic SD (RSD[1]) for shear force and the tenderness score, with the corresponding *P*-values, for the Blonde d'Aquitaine breed

	Shear force				Tenderness score			
Haplotype	a ± SE	a/RSD		*P*-value	a ± SE	a/RSD		*P*-value
CAST								
AGG	−1.39 ± 1.96	−0.13	ab	0.48	−1.07 ± 2.13	−0.10	ab	0.61
GGG	−0.73 ± 0.90	−0.07	a	0.42	−0.58 ± 0.96	−0.05	ab	0.55
AAG	−0.72 ± 0.87	−0.07	a	0.41	1.47 ± 0.92	0.14	a	0.11
GAA	−0.55 ± 0.64	−0.05	a	0.39	0.55 ± 0.69	0.05	a	0.42
GAG	0.29 ± 1.88	0.03	ab	0.88	2.37 ± 2.03	0.22	a	0.24
GGA	3.10 ± 0.83	0.30	b	0.0002	−2.74 ± 0.88	−0.26	b	0.002
CAPN1								
GGGA	−1.37 ± 0.59	−0.13	a	0.02	1.23 ± 0.62	0.11	a	0.05
GGAG	−1.04 ± 0.59	−0.10	a	0.08	0.47 ± 0.61	0.04	ab	0.44
AGAG	−0.72 ± 1.14	−0.07	ab	0.53	1.13 ± 1.20	0.11	ab	0.35
AGGG	−0.36 ± 1.24	−0.04	ab	0.77	−0.04 ± 1.27	−0.004	ab	0.97
ACGA	0.69 ± 1.43	0.07	ab	0.63	−0.88 ± 1.51	−0.08	ab	0.56
GGGG	0.80 ± 0.66	0.08	b	0.23	−0.01 ± 0.70	−0.001	ab	0.99
AGGA	2.01 ± 1.05	0.19	b	0.06	−1.90 ± 1.10	−0.18	b	0.09

[a,b]For each trait, haplotypes without a common letter differ ($P < 0.05$).
[1]RSD: root mean square error of the basic model that includes only the fixed effect of the contemporary groups.

but not in the pure French Charolais, Limousin, and Blonde d'Aquitaine breeds. In the present study, we estimated a G allele frequency of about 20% in the 3 French breeds, which was similar to that estimated in the *Bos taurus* crossbred GPE Cycle VII and *B. taurus* × *B. indicus* crossbred Cycle VIII populations (Casas et al., 2006) but less than in the *B. indicus* Brahman population (between 28 and 43%; Van Eenennaam et al., 2007; GeneNote 4, 2010; Johnston and Graser, 2010) and in the CRC1 population of pooled tropical breeds (Brahman, Santa Gertrudis, and Belmont Red: 29%; Johnston and Graser, 2010). The G allele frequency calculated in a crossbred Charolais × Angus population was particularly small (0.06%; Van Eenennaam et al., 2007), whereas it was estimated at 12% in the pure Angus breed (GeneNote 4, 2010; Johnston and Graser, 2010). Johnston and Graser (2010) found a 13% G allele frequency in the CRC1 population of temperate breeds (Angus, Hereford, Murray Grey, Shorthorn). Regarding the *CAPN1* gene, we found that the G allele of the *CAPN1–2* marker had greater frequencies in the Charolais and Blonde d'Aquitaine breeds (91 and 96%, respectively) and a decreased frequency in the Limousin breed (73%). Page et al. (2004) estimated the G allele frequency of this marker in the Charolais and Limousin breeds on the basis of purebred sires in the GPE cycle VII population. Page et al. (2004) found frequencies of 95% in the Charolais breed and 92% in the Limousin breed. These frequencies may have been overestimated by Page et al. (2004) because the small number of bulls limited the possibility for accurate estimates. Casas et al. (2005) and Johnston and Graser (2010) estimated a very high G allele frequency in the Brahman breed (97%), and White et al. (2005) found that this marker was not informative in a Hereford × Brahman population. The same authors estimated that the G allele frequencies were 80 and 83% in the GPE cycle VII and cycle VIII populations, respectively. Johnston and Graser (2010) found that the G allele frequencies were the same in the 2 CRC1 populations (about 70%), but less in the Angus subpopulation (57%). The *CAPN1–3* marker has also been well described in the literature. We found a G allele frequency of 76% in the Charolais breed and 64% in the Limousin and Blonde d'Aquitaine

Table 12. Additive effects of each haplotype of markers 2 and 3 of the calpastatin (*CAST*) gene in the Blonde d'Aquitaine breed with SE, divided by the phenotypic SD (RSD[1]) for shear force and the tenderness score, with the corresponding *P*-values

		Shear force				Tenderness score			
Haplotype	n	a ± SE	a/RSD		*P*-value	a ± SE	a/RSD		*P*-value
GG	181	−1.09 ± 0.67	−0.10	a	0.10	−0.29 ± 0.71	−0.03	ab	0.69
AG	265	−0.85 ± 0.62	−0.08	a	0.17	1.81 ± 0.65	0.17	a	0.01
AA	1,220	−0.83 ± 0.41	−0.08	a	0.04	0.84 ± 0.43	0.08	a	0.05
GA	262	2.77 ± 0.61	0.27	b	<0.001	−2.37 ± 0.64	−0.22	b	0.0002

[a,b]For each trait, haplotypes without a common letter differ ($P < 0.05$).
[1]RSD: root mean square error of the basic model that includes only the fixed effect of the contemporary groups.

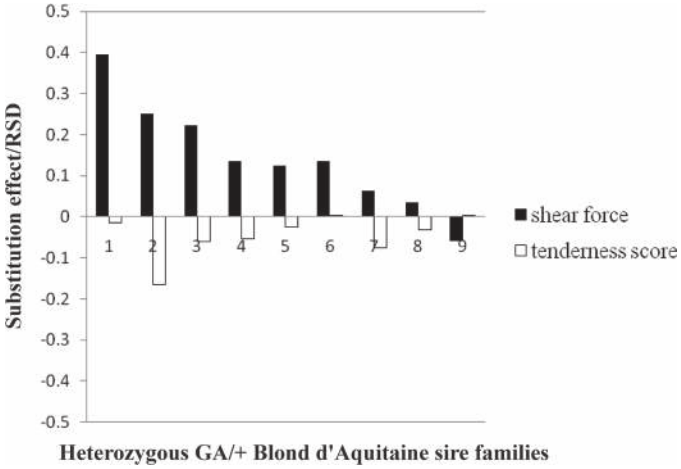

Figure 1. Substitution effect of a haplotype (+) by the GA calpastatin (*CAST*) haplotype, divided by the phenotypic SD (RSD) for shear force and the tenderness score, in each heterozygous Blonde d'Aquitaine sire family.

breeds. Casas et al. (2005) showed that the G allele was fixed in the Brahman breed. White et al. (2005) described the same monomorphism in a Brahman × Hereford population. The same authors estimated a G allele frequency of 86% in the GPE Cycle VIII population. Page et al. (2004) found a G allele frequency of 78% in the GPE Cycle VII population. The 4 other SNP (2 in the *CAPN1* gene and 2 in the *CAST* gene) genotyped during the present study were original, and no results could be found in the literature.

CAST Gene Associations with Meat Tenderness

Casas et al. (2006) found significant additive effects of the G allele of *CAST-2* on shear force and the tenderness score in the GPE cycle7 crossbred *B. taurus* population. These effects were in the same order as those found by us in the Blonde d'Aquitaine breed (0.20 SD). Johnston and Graser (2010) found the same effect on shear force in the CRC1 population of pooled temperate breeds, but no effect in the Shorthorn subpopulation. Johnston and Graser (2010) showed that the G allele exerted effects of 0.17 SD in the CRC1 population of pooled tropical breeds and 0.13 SD in a Brahman population. In their validation study, Van Eenennaam et al. (2007) found a reduced G allele effect (+0.10 SD on shear force) in a population of Charolais × Angus, Hereford, and Brahman animals. We could not determine any effect of this marker in the Charolais and Limousin breeds during our study. We analyzed 2 other polymorphisms in the CAST gene, and the *CAST-1* and *CAST-3* markers only had an effect on 1 trait in the Limousin and Charolais breeds, respectively. We were more confident in our results if a marker had an effect on both traits, especially if this effect was weak. The study of haplotypes revealed a very interesting GA haplotype on the *CAST-2* and *CAST-3* markers in the Blonde d'Aquitaine breed. The GA haplotype, specific to the Blonde d'Aquitaine breed, was associated with tough meat (+0.27 SD on shear force and −0.22 SD on the tenderness score) at the population level and within sire families. In a context of developing a commercial test, a reduction in the number of markers that need to be genotyped is of considerable importance to breeding companies.

CAPN1 Gene Associations with Meat Tenderness

Page et al. (2004) revealed an association between genotypes at the *CAPN1–2* and *CAPN1–3* markers and shear force in a Simmental × Angus ASA population and in the GPE Cycle VII *B. taurus* crossbred population. In the ASA population, Page et al. (2004) found additive effects of the G allele of +0.37 SD for the *CAPN1–2* marker and −0.22 SD for the *CAPN1–3* marker. In the Cycle VII population, these effects were weaker (+0.18 SD for the *CAPN1–2* marker and −0.12 SD for the *CAPN1–3* marker). White et al. (2005) showed that the G allele of the *CAPN1–2* marker was associated with shear force in the GPE Cycle VIII *B. indicus* × *B. taurus* crossbred population (+0.38 SD). During our study, the *CAPN1–3* marker did not display a significant association with shear force and the tenderness score in the 3 French breeds. The *CAPN1–3* marker had not been found to affect shear force in the GPE Cycle VIII population (White et al., 2005). As for the *CAPN1–2* marker, we showed that the A allele segregated at a low frequency in the Charolais breed (9%). However, we found an association between this marker and the 2 traits in this breed. The G allele exerted a negative effect on meat tenderness (+0.22 SD of shear force and −0.25 SD on the tenderness score), which is consistent with the results obtained by Page et al. (2004) and White et al. (2005). This effect was greater than those found by Johnston and Graser (2010) on shear force in the CRC1 populations (+0.11 SD in the population of pooled temperate breeds and +0.18 SD in population of pooled tropical breeds). In the Limousin breed, we only found a G allele effect on shear force (+0.12 RSD). There was no effect in the Blonde d'Aquitaine breed, but the A allele segregated at a decreased frequency (4%) in this breed. Two other polymorphisms were also analyzed during this study. The *CAPN1–1* marker in exon 6 did not display an association with meat tenderness, but we found an interesting association between the *CAPN1–4* marker and meat tenderness in the Charolais breed. Haplotype analysis revealed 2 antagonist haplotypes in the Charolais breed: the ACA haplotype on the *CAPN1–1*, *CAPN1–2*, and *CAPN1–4* markers was associated with tender meat and the AGG haplotype was associated with tough meat at the population level.

In conclusion, this study focused on some of the published and original markers found in the *CAST* and *CAPN1* genes that segregate in French beef breeds. We found some SNP and haplotype effects on meat ten-

derness traits, which were breed-specific. Consequently, the current work demonstrates that the effects of these markers cannot be extended to all *B. taurus* breeds. Additional work may be required to test other markers included in commercial genetic tests. For example, the C/T polymorphism at the position 6545 of GenBank Accession No. AF248054 in the *CAPN1* gene might be of particular interest because an association has been found between this marker and meat tenderness in *B. taurus* and *B. indicus* populations (White et al., 2005; Casas et al., 2006; Van Eenennaam et al., 2007; Curi et al., 2009), as well as the G/C polymorphism in the intronic sequence between exons 5 and 6 of the *CAST* gene (base 282 of GenBank Accession No. AY008267; Schenkel et al., 2006). These 2 markers are included in American or Australian commercial genetic tests. However, more promising results may be obtained from fine mapping the whole genome with a high-density SNP chip to discover new markers in linkage disequilibrium with tenderness QTL, which together may explain a significant proportion of the genetic variance observed in these 3 beef breeds.

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