Association of single nucleotide polymorphisms in *CAPN1* and *CAST* genes with beef tenderness from Spanish commercial feedlots

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ABSTRACT: Frequencies of two SNPs in the μ-calpain (*CAPN1*) and calpastatin (*CAST*) genes in local and foreign commercial cross-breeds used in south-western Spain (Charolais, Limousin, and Retinta) were evaluated and the association of these markers with texture analysis in animals fattened under different feedlot conditions was assessed. Marker frequencies were estimated in a 286 bull crossbred population and the *longissimus dorsi* muscles from subsequently selected 161 animals were used to measure Warner-Bratzler shear force in raw and cooked samples at three different ageing days (1, 7, and 21). Significant differences ($P \leq 0.05$) were found for shear force in raw and cooked meat samples for the three ageing days for the three crossbreeds analyzed. Significant associations were observed for raw meat for the Charolais between shear force and the *CAPN1* marker ($P = 0.019$), as well as between the *CAST* polymorphism and shear force ($P = 0.027$) in the Limousin. No associations were found between the markers and shear force in the Retinta ($P > 0.05$). In contrast, although these markers might be useful in particular selected populations due to their effect on objective texture parameters, no significant association ($P > 0.05$) was found for cooked meat in the sample of Spanish commercial crossbreeds used in this study. Further studies with a higher number of animals will be necessary to confirm these results.

Keywords: association studies; *Bos taurus*; calpain; calpastatin; meat tenderness; molecular markers; shear force; SNP

The dehesa of the south-western Iberian Peninsula is a "man-made" ecosystem characterized by a savannah-like physiognomy (Joffre et al., 1999). Breed crosses between local (Retinta) and foreign (mainly Charolais and Limousin) breeds are reared on these territories characterized by highly variable Mediterranean climate. The crossbreeds maintain the high adaptation capacity to natural conditions from the local breeds while they acquire a productive potential from the foreign breeds.

Production or handling factors, including breed, age, sex, feeding, and pre-slaughter management, can determine the potential quality of meat characteristics (Monsón et al., 2004). Inadequate tenderness is the most serious cause of consumers' dissatisfaction and any improvement in tenderness would increase the value of the final product (Brooks et al., 2000). In fact, decreasing of this variability is one of the main current objectives of the meat industry because of its concern to homogenize the products to suit different markets (Warner et al., 2010).

Ageing has been reported to be the most important factor influencing beef tenderness (Juárez et

al., 2011). Warner-Bratzler shear force is one of the standard tools to quantify tenderness because it presents a negative correlation with initial tenderness (–0.61), amount of perceptible connective tissue (–0.49), and overall tenderness (–0.60) assessed by a trained sensory panel (Caine et al., 2003). Among the factors responsible for the postmortem meat tenderization during ageing, the calpain-calpastatin proteolytic system has been identified to play a key role (Koohmaraie and Geesink, 2006). Two enzymes responsible for this process are the micromolar calcium-activated neutral protease μ-calpain, and its inhibitor, calpastatin (Koohmaraie, 1996). The moderate heritability (h^2) of tenderness (0.14–0.47) (O'Connor et al., 1997; Dikeman et al., 2005; Wheeler et al., 2005; Boukha et al., 2011) represents a potential for improvement through animal selection in a breeding program. However, tenderness cannot be measured routinely in commercial conditions, so Marker Assisted Selection is considered as a good alternative to improve the trait.

Several single nucleotide polymorphisms (SNP) in the μ-calpain (*CAPN1*) and calpastatin (*CAST*) genes have been reported to be associated with tenderness in beef cattle. Two markers in the *CAPN1* and *CAST* genes previously reported (Page et al., 2002; Schenkel et al., 2006) take part in the composition of a commercial DNA test and have been validated by Van Eenennaam et al. (2007). The SNP referred to as *CAPN1* is located on BTA29. The marker, known as *CAPN1-316*, is a transversion from guanine to cytosine at position 5709 of the GenBank Accession No. AF252504. The SNP referred to as *CAST* (mapped to BTA7) is also a guanine to cytosine transversion at position 282 of the GenBank Accession No. AY008267. The *C* allele of both markers is associated with more tender meat.

The effects of the markers tend to be breed-specific and cannot be extended to all *Bos taurus* breeds (Allais et al., 2011) without a detailed analysis for each particular population. There are few association studies of markers related to meat tenderness in local beef breeds and their crosses with foreign breed populations reared in Spain (Avilés et al., 2007). Moreover, different feedlot production systems may also have a significant impact on the expression of those genes.

The aim of this study was to evaluate the frequencies of two SNPs in the *CAPN1* and *CAST* genes in local and foreign commercial crossbreeds used in south-western Spain and to assess the association of these markers with texture analysis in animals fattened under different feedlot conditions.

MATERIAL AND METHODS

Experimental design and sample collection

The experiment was developed in two replicates: year 1 (n = 137) and year 2 (n = 149). Two hundred eighty-six crossbred cows with a genetic basis of the main breed reared in the south-western Spain, the Retinta (RE), and a different level of Charolais (CH) or Limousine (LI) blood were mated to CH, LI, and RE purebred sires. The entire males of the F_1 population were originally selected from 20 different farms (in order to achieve the maximum variability of the population) based on morphological characteristics. The breed crosses and the number of individuals per cross were as follows: CH n = 98, LI n = 99, and RE n = 89. The animals were allocated to two different commercial diets (feedlot types): a mixture of concentrate, corn silage, straw, and beetroot pulp (type 1: 49 for CH, 49 for LI, and 51 for RE) and a conventional feeding diet of straw plus concentrate (type 2: 49 for CH, 50 for LI, and 38 for RE). The bulls were slaughtered when they reached approximately 550 kg of live weight (mean ± SD = 544 ± 35.3) in a commercial abattoir according to the Council Directive 93/119/EC (1993). Carcasses were chilled at 4°C for 24 h with a constant air velocity of 0.5 m/s and a relative humidity of 90%.

DNA extraction and genotyping

Blood samples (5 ml) were collected from the caudal vein of 286 entire males belonging to three crossbred types. Genomic DNA was isolated from aliquots of 200 μl of blood samples using a commercial kit (Dominion®, Dominion-MBL s. l., Cordoba, Spain) according to manufacturer's instructions. The quality and amount of DNA was measured using a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Genomic DNA of each animal was stored at –20°C until subjected to allelic discrimination assays.

Amplification and genotyping of the *CAPN1* (SNP AF252504:g.5709G>C) and *CAST* (SNP AY008267:g.282G>C) genes was carried out using the 5' nuclease allelic discrimination assay.

Table 1. Primer and probe sequences (5' to 3') for each genotyped polymorphism

	CAPN1	*CAST*
Forward primer	gcagtgccgttttcctacag	ctgaatttggaaggaaggaattgca
Reverse primer	agctgctcccgcatgtaag	caattgtgagaatttaaattagtatgatttacatgtgaca
Probe 1[a]	tccacg**g**cgttcca	tttgggta**g**aaaattt
Probe 2	tccacg**c**cgttcca	ttgggta**c**aaaattt

[a]probe nucleotides in bold target the specific alternative alleles of a particular SNP

Samples were screened on an ABI PRISM® 7500 FAST Real Time system (Applied Biosystems, Foster City, USA) from the UCO-SCAI genomics facility. Primers and probes (with a different reporter dye on each probe) were designed (Table 1) by the manufacturer using the sequences previously analyzed by direct sequencing (see Avilés et al. (2009) for more details).

A panel of 17 microsatellites was used to assign each animal to its own population with the GeneClass (Version 1.0.02, 1999) software. The probability of correct assignation was at least 75% with a maximum of 90% (mean = 81.8%). One hundred and sixty-one animals (54 for CH, 55 for LI, and 52 for RE) from the original 286 bull population were selected for subsequent meat quality analyses (animals wrongly assigned to the populations by morphological characteristics were then rejected).

Meat quality

In terms of the EEC Beef Carcass Classification Scheme, Commission Regulation (EU) No. 1249/08/EC (EU Commission Regulation, 2008), all carcasses fell within the class 2 for fatness and R+ to U– for conformation. The values of pH and temperature at the centre of the *longissimus dorsi* muscle were assessed at 20 min and 24 h post-mortem in order to detect carcasses with potential cold shortening risk (pH > 6.0 at temperatures < 10–12°C) or non-favourable ultimate pH (5.8–6.2). The *longissimus dorsi* muscle (between T6 and L6) was removed from the left carcass side 24 h post-mortem and sliced into 2 cm steaks. Three pairs of steaks, balanced by location, were vacuum packaged and stored at 4°C until they were subjected to shear force analysis after 1, 7, and 21 days of ageing.

Both steaks were used either as raw or cooked meat samples. Meat was cooked in a clamshell grill (PL. 4 model, Ascaso Factory, Barcelona, Spain) at 190°C until the internal temperature measured by HI98509 Checktemp®1C Pocket Thermometer (Hanna Instruments, Woonsocket, USA) reached 71°C. Six cores per steak (raw and cooked), with 1 cm^2 in cross section, were cut with muscle fibres parallel to the longitudinal axis of the sample. The cores were tested with a TA-XT2 instrument (Stable Micro Systems, Godalming, UK) using the Warner-Bratzler shearing device (crosshead speed 200 mm/m). The average shear force (for each steak amount of force necessary to completely cut the core) expressed in kg/cm^2 was reported as the average value for all evaluated cores.

Statistical methods

Genotype and allele frequencies were calculated for the genotyped sample set (n = 286). To assess the Hardy-Weinberg equilibrium, the total sample set and each crossbreed were analyzed using the exact probability test in GENEPOP (Raymond and Rousset, 1995a). Pairwise tests for genic differentiation (Raymond and Rousset, 1995b) were carried out to establish if the allelic frequencies were significantly different ($P \leq 0.05$) among breeds.

The data to compare the repeated shear force measurements for each genotype of the *CAPN1* and *CAST* genes across the 3 ageing days in raw and cooked meat were tested using a linear mixed effect model through the MIXED Procedure of SAS (Statistical Analysis System, Version 9.2, 2008). An unstructured covariance structure was used assuming that the variability within ageing and the correlation among the three days are not homogeneous. The model was as follows:

$$y_{ijk} = \mu + G_i + A_j + F_k + (G \times A)_{ij} + (G \times F)_{ik} + (A \times F)_{jk} + \\ + (G \times A \times F)_{ijk} + \beta_{SW} + e_{ijk}$$

Separate analyses were carried out for each SNP and breed due to the significant differences ($P \leq 0.05$) of the breed × genotype interaction found in the previous model where the breed was included

as a fixed effect. For each marker, μ is the general mean of the trait, genotype (G_i), ageing day (A_j) and feedlot type (F_k) were included as fixed effects. Year was treated as a random effect. Second order interactions between effects were included. Slaughter weight was included as a linear covariate (β_{SW}), e_{ijk} is the residual error. Mean comparison was performed using the LSMEANS option using *F*-test protected LSD ($P \leq 0.05$). Quadratic contrasts were conducted to further explore the effect of genotype and ageing time on beef texture. A linear regression on the number of *C* alleles (0, 1, and 2) with the same models to calculate the genotype effects was used to estimate the average allele substitution effects.

RESULTS AND DISCUSSION

Allelic and genotypic distribution

The first objective of this study was to assess the frequencies of two SNPs in the *CAPN1* and *CAST* genes in local and foreign commercial crossbreeds commonly used by the beef industry in southwestern Spain. These markers, which are included in commercial tests (such as GeneSTAR® Tenderness or Igenity® Tender-GENE), have been developed in previous works by examining populations that incorporate a wide variety of commercial crossbreeds.

Allele and genotype frequencies of each crossbreed are provided in Table 2. Significant deviations from the Hardy-Weinberg proportions were observed for the *CAPN1* marker ($P < 0.01$). However, no significant differences were found for the *CAST* marker ($P > 0.05$).

Similar allelic frequencies for the CH (*C* allele frequency = 0.36) and the LI (*C* allele frequency = 0.32) crossbreeds were found for the *CAPN1* marker. On the other hand, significant differences regarding the allelic frequencies of the RE crossbreed (*C* allele frequency = 0.64) were detected and pairwise tests for genic differentiation among crossbreeds confirmed that RE–LI ($P < 0.01$) and RE–CH ($P < 0.01$) for the *CAPN1* locus had significantly divergent allele frequencies. Page et al. (2004) estimated the *C* allele frequency in CH (0.05) and LI (0.08) breeds. However, Allais et al. (2011) found higher frequencies for the same allele, 0.09 in the CH and 0.27 in the LI purebred populations. Regarding the RE crossbreed, Avilés et al. (2009) estimated a frequency of 0.29 for the *C* allele.

The allelic distribution for the *CAST* marker in the three populations followed a different pattern. The *C* allele frequencies were 0.76 for the CH, 0.65 for the LI, and 0.67 for the RE crossbreeds. In the pairwise tests for genic differentiation among crossbreeds, the comparison between the LI and the CH breeds showed a significant difference of the allele frequencies for the *CAST* locus ($P = 0.035$), as well as a trend ($P = 0.092$) towards significance for RE–CH.

Schenkel et al. (2006) estimated the *C* allele frequencies in CH (0.69) and LI (0.73) crossbreeds. The small number of animals belonging to CH (8) and LI (28) breeds used by Schenkel et al. (2006) most likely contributed to the differences observed regarding the results here presented. For the RE crossbreed, the *C* allele frequency (0.67) was lower than that found in a preliminary study (0.91) (Avilés et al., 2007), where a population belonging to the Retinta's Herdbook was used. This variability might be attributed to the difference in the genotype (crossbred vs. purebred). Retinta breed is classified within the Red Convex Branch of cattle in the Iberian Peninsula and it has a high African influence (Pellecchia et al., 2007). This might explain the differences with crossbreeds from French origin beef cattle breeds.

Phenotypic traits

The value of pH measured 24 h post slaughter in the centre of the *longissimus dorsi* muscle was

Table 2. Genotype and allelic frequencies for the *CAPN1* and *CAST* loci for the three crossbred cattle populations genotyped

Breed	*CAPN1*					*CAST*				
	CC	*CG*	*GG*	*C*	*G*	*CC*	*CG*	*GG*	*C*	*G*
Charolais ($n = 98$)	0.26	0.21	0.53	0.36	0.64	0.56	0.39	0.05	0.76	0.24
Limousin ($n = 99$)	0.26	0.12	0.62	0.32	0.68	0.41	0.48	0.11	0.65	0.35
Retinta ($n = 89$)	0.59	0.11	0.30	0.64	0.36	0.43	0.49	0.08	0.67	0.33

Table 3. Probability of the *F*-test for genotype, ageing, and feedlot effects and interactions on shear force values for each crossbreed tested (*P*-values)

	Charolais		Limousin		Retinta	
	SFR	SFC	SFR	SFC	SFR	SFC
μ-Calpain marker						
CAPN1	4.01*	0.36^{ns}	1.80^{ns}	2.38^{t}	0.33^{ns}	0.11^{ns}
Day	5.98**	56.38***	4.49*	176.01***	13.01***	128.46***
Feedlot	0.12^{ns}	7.11**	0.98^{ns}	0.02^{ns}	1.47^{ns}	8.12**
CAPN1 × day	0.99^{ns}	1.41^{ns}	1.39^{ns}	0.75^{ns}	2.01^{t}	0.14^{ns}
CAPN1 × feedlot	0.68^{ns}	0.36^{ns}	1.29^{ns}	1.86^{ns}	1.02^{ns}	1.87^{ns}
Day × feedlot	0.63^{ns}	3.85*	1.82^{ns}	1.03^{ns}	0.04^{ns}	0.86^{ns}
CAPN1 × day × feedlot	0.23^{ns}	1.38^{ns}	0.05^{ns}	0.18^{ns}	1.68^{ns}	0.27^{ns}
Calpastatin marker						
CAST	2.58^{t}	1.58^{ns}	3.95*	1.08^{ns}	0.70^{ns}	1.92^{t}
Day	10.22***	37.80***	3.43*	66.17***	9.42***	92.63***
Feedlot	2.89^{t}	18.97***	1.97^{ns}	5.75*	0.12^{ns}	3.20^{t}
CAST × day	2.28^{t}	0.52^{ns}	0.57^{ns}	0.84^{ns}	0.88^{ns}	0.56^{ns}
CAST × feedlot	0.08^{ns}	0.67^{ns}	3.10^{t}	1.29^{ns}	0.99^{ns}	0.94^{ns}
Day × feedlot	3.57*	5.20**	1.92^{t}	0.87^{ns}	0.43^{ns}	0.33^{ns}
CAST × day × feedlot	0.89^{ns}	0.38^{ns}	0.31^{ns}	0.92^{ns}	0.77^{ns}	0.68^{ns}

SFR = shear force in raw meat, SFC = shear force in cooked meat

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; $^{t}P < 0.1$, ns = nonsignificant differences

confidence level of predicted factors ($P \leq 0.05$)

under 5.8 for all the carcasses. Significant differences ($P \leq 0.05$) were found for shear force in raw (SFR) and cooked (SFC) meat among ageing days for the three crossbreeds analyzed (Table 3); no interaction ($P > 0.05$) was observed between ageing day and genotype for any of the markers.

Shear force values for raw and cooked meat from ageing days 1–21 in the three crossbred groups are given in Figure 1. The SFR did not present the same behaviour on the three populations. The CH and the RE crossbreeds showed higher values ($P \leq 0.05$) on day 1 compared to day 7, and kept the same shear force until day 21, meanwhile no significant differences ($P > 0.05$) were observed in SFR for the LI crossbreed between days 1, 7, and 21. However, the quadratic trend was more remarkable in SFR for the RE than for the CH and the LI crossbreeds. Lower SFR were observed than those reported by Christensen et al. (2011) for CH and LI breeds. No previous reports have been found about SFR in RE breed or crossbreeds.

The values observed for SFC in the present study were higher for the CH and lower for the LI crossbreeds than those reported by Christensen et al. (2011) for French CH and LI breed populations. A higher SFC was obtained for the RE crossbreed than that observed in a previous study (Sañudo et al., 1998). All crossbreeds started at a similar shear force level on ageing day 1 in cooked meat (10.2, 10.5, and 10.4 kg/cm^2 for CH, LI, and RE, respectively) but the decrease between days 1–16 was higher in the LI than in the CH and the RE breed types. However, from day 16 to day 21, although the SFC in the three crossbreeds continued decreasing ($P < 0.01$), it showed a quadratic trend for the LI crossbreed. Ageing time was shorter in the LI crossbreed than in the CH and the RE ones. Monsón et al. (2004) found LI breed was the most tender in the early ageing period comparing four different biotypes and recommended to consume the meat of this breed at short ageing times. Juárez et al. (2010) reported a quadratic trend of tenderness after extended ageing time due to, among other factors, moisture loss and its effect on cooking parameters. This tendency was also observed in the present study.

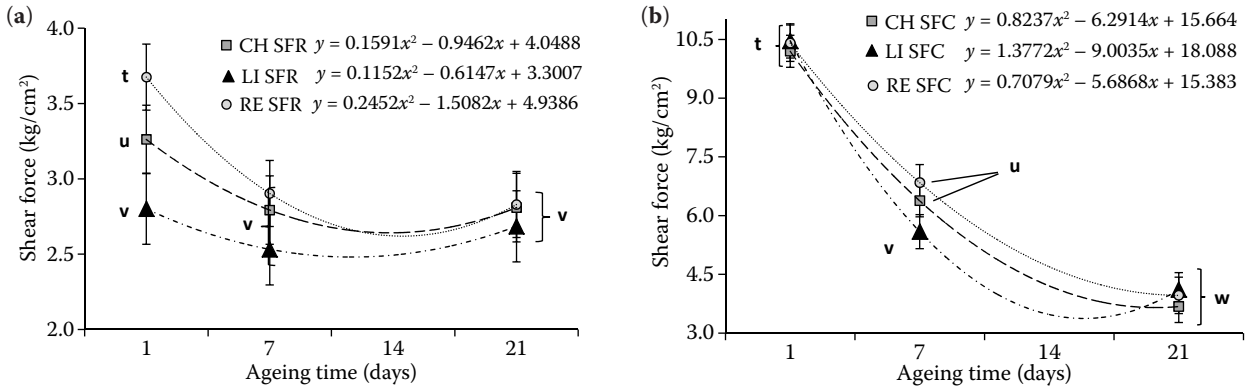

Figure 1. Shear force values for raw (**a**) and cooked (**b**) meat from ageing days 1–21 in the three crossbred groups

CH = Charolais, LI = Limousin, RE = Retinta

$^{t–w}$different letters indicate significant differences ($P < 0.05$) among days and/or crossbreeds

All the phenotypic differences among populations could be related to the genetic differences in the enzymatic activity of muscle and in its chemical-biological composition, differences in the intramuscular fat levels or differences in fibre typology, as reported by Sañudo et al. (2004). Shear force in raw beef has a positive relationship with total and insoluble collagen contents (Torrescano et al., 2003), in contrast to shear force in cooked meat, where the tenderization process is related to several aspects, among which myofibrillar degradation stands out (Uytterhaegen et al., 1994). Moreover, shear force differences among crossbreeds could be also explained by variability in the genotype composition and by the fact that purebred animals were not used in the present study.

Forage finishing of cattle has been shown to have negative consequences on meat tenderness. French et al. (2000) found that animals fed with a diet based on grass dry matter plus low levels of concentrate had lower Warner-Bratzler shear force than those fed with a silage plus concentrate diet, after 2 days of ageing. This difference was not found for ageing days 7 and 14.

CH and LI breeds are considered late-maturing. RE is an intermediate-maturing breed not as highly selected for muscular performance or carcass classification score as late-maturing breeds. These differences were reflected in fatness and leanness and hence, in meat characteristics. CH bulls were found to be heavier than LI ones. The differences in several other parameters related to fibre type, collagen contents, and enzymatic activities have also been reported (Jurie et al., 2005). This might explain why only meat from the CH crossbreed showed a different behaviour pattern when the diet changed. The interaction between ageing day and feedlot in the CH crossbreed showed significant differences ($P \leq 0.05$) for the effect of both markers on SFC and for the *CAST* SNP effect on SFR. Animals coming from feedlot 2 were more tender than those coming from feedlot 1 on day 1 (8.50 vs. 11.64 kg/cm^2; $P < 0.01$), however this difference became lower overtime and was not appreciable on days 7 and 21 ($P > 0.05$). The results in the present study showed the same pattern as those previously reported by other authors (French et al., 2000; Jurie et al., 2005). Changes in pH due to diet 24 h post slaughter might be behind the differences observed between feedlots.

Marker associations

Genotype Least Squares Means, standard errors, *P*-values, and average allele substitution effects of the *CAPN1* and *CAST* markers on SFR and SFC in the populations studied are reported in Table 4. The interaction between genotype and ageing times was not significant ($P > 0.05$).

In contrast to cooked meat, there are not previous association studies between raw meat and the *CAPN1* or *CAST* markers, since SFR has been used to detect collagen content variations. Nevertheless, significant associations ($P \leq 0.05$) were observed in the CH type between SFR and the *CAPN1* marker ($P = 0.019$) as well as between the *CAST* SNP and SFR ($P = 0.021$) in the LI crossbreed. The *CC* genotype of the *CAPN1* marker in the CH crossbreed as well as the *CAST* marker in the LI crossbreed

Table 4. μ-Calpain and calpastatin SNP effect on shear force in raw and cooked meat of the three crossbreeds

	Charolais		Limousin		Retinta	
	SFR	SFC	SFR	SFC	SFR	SFC
CAPN1						
CC	2.64 ± 0.30^{y}	6.72 ± 0.51	2.35 ± 0.18	6.57 ± 0.25	3.13 ± 0.16	6.94 ± 0.48
CG	3.10 ± 0.31^{x}	6.72 ± 0.59	2.34 ± 0.20	7.53 ± 0.39	3.28 ± 0.29	7.28 ± 0.64
GG	2.94 ± 0.29^{x}	6.98 ± 0.45	2.52 ± 0.17	6.81 ± 0.19	3.23 ± 0.20	6.83 ± 0.52
Average allele substitution effect ± SE (kg/cm$^{2)}$	–0.14 ± 0.06	–0.13 ± 0.20	–0.08 ± 0.05	–0.11 ± 016	–0.06 ± 0.09	0.03 ± 0.16
P-value	0.027	0.507	0.088	0.472	0.510	0.862
CAST						
CC	2.80 ± 0.29	6.68 ± 0.41	2.31 ± 0.15^{y}	6.70 ± 0.22	3.22 ± 0.18	6.60 ± 0.46
CG	3.05 ± 0.30	7.23 ± 0.45	2.57 ± 0.15^{x}	7.15 ± 0.22	3.19 ± 0.18	7.20 ± 0.47
GG	2.78 ± 0.47	7.44 ± 1.22	2.44 ± 0.18^{xy}	6.19 ± 0.39	2.82 ± 0.28	7.03 ± 0.60
Average allele substitution effect ± SE (kg/cm$^{2)}$	–0.19 ± 0.10	–0.51 ± 0.31	–0.11 ± 0.06	0.08 ± 0.20	0.14 ± 0.12	–0.35 ± 0.21
P-value	0.060	0.100	0.067	0.706	0.245	0.097

SFR = shear force in raw meat (Least Squares Means ± SE), SFC = shear force in cooked meat (Least Squares Means ± SE)
$^{x–y}$different letters within breed indicate significant differences among genotypes
confidence level of predicted factors ($P \leq 0.05$)

were associated with more tender raw meat. The allele substitution effect for the trait estimated by the repeated measures analysis was –0.14 ± 0.06 for the CH crossbreed and *CAPN1* marker, and –0.11 ± 0.06 for the same trait between LI crossbreed and *CAST* marker. As expected, associations between shear force and markers dropped after cooking. Structural and distribution changes experimented by meat in factors such as collagen or water contents (Aalhus et al., 2009) after cooking diluted the effect of the myofibrillar component on the original texture characteristics. Warner-Bratzler shear force method can lose its ability to discriminate slight differences in shear force. Sensory panel or myofibrillar degradation index analyses could be more accurate to detect these associations with the *CAPN1* and *CAST* markers. No associations ($P > 0.05$) were found between raw meat and markers in the RE crossbreed.

No significant associations ($P > 0.05$) were found between SFC and the *CAPN1* marker, although there was a trend towards significance ($P = 0.076$) for the LI crossbreed, where *CC* genotype was related to more tender meat, as reported by Page et al. (2004). For the *CAST* SNP, no significant associations were observed between SFC and the marker although, in line with results obtained by Schenkel et al. (2006) and Van Eenennaam et al. (2007), the allele substitution effect in the RE crossbreed presented a trend towards significance ($P = 0.097$) with increased tenderness in beef from genotype *CC* vs. *CG* and *GG* (–0.60 and 0.43 kg/cm^2, respectively).

CONCLUSION

Significant associations were found between shear force and *CAPN1* and *CAST* markers in raw meat from commercial crossbreeds commonly used in south-western Spain. However, no significant associations were found between the *CAPN1* and cooked meat. Only the *CAPN1* marker in the LI crossbreed presented a trend towards significance for cooked meat. Consequently, samples were more homogeneous after cooking and associations disappeared. There are not strong associations between markers and shear force in the RE crossbreed probably because the RE purebred has not been highly selected and, as a result, its population is more heterogeneous in a wide range of different traits, such as tenderness. Moreover, the effects of marker tend to be breed-specific and these association studies have never used RE breed. Further studies with a higher number of

animals will be necessary to confirm these results in Spanish commercial crossbreeds.

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