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Association between molecular markers for beef tenderness and growth traits in Argentinian Angus cattle

D. Pintos and P. M. Corva

Department of Animal Science, College of Agricultural Sciences, University of Mar del Plata, CC 276, 7620 Balcarce, Argentina

Summary

Molecular markers for beef tenderness are classic examples of the contribution of genome technology to animal breeding through marker-assisted selection (MAS). Markers on the *μ-calpain (CAPN1)* and *calpastatin (CAST)* genes have been extensively evaluated for their association with tenderness. However, little is known about their potential effect on other economically important traits. In this work, the association of molecular markers for beef tenderness with growth traits was evaluated in Angus cattle of Argentina. Expected progeny differences were extracted from the 2008 Angus Sire Summary of Argentina. Information corresponding to 268 influential bulls that had been genotyped for two markers in *CAPN1* and two markers in *CAST* was provided by the Argentine Angus Association. Genotype probabilities were assigned, by segregation analysis, to those bulls in the Sire Summary that had no marker information. Expected progeny differences of 1365 sires were regressed on the number of alleles favouring tenderness at each locus. There was a significant effect of markers on expected progeny differences of birth weight, weaning weight (direct), weight at 18 months and rib eye area. In general, there was a negative effect of alleles favouring tenderness on growth traits. These correlated responses should be taken into account when molecular markers are used in selection schemes that aim to improve beef tenderness.

Keywords μ-calpain, Angus, *calpastatin*, expected progeny difference, growth, molecular markers.

Tenderness is one of the attributes of quality of beef that is most appreciated by consumers (Alfnes *et al.* 2008; Verbeke *et al.* 2010); however, it is usually not included in animal breeding programs because of the inherent difficulties of measuring this trait under commercial conditions. Given the relevance of the trait, molecular markers for beef tenderness were part of the first commercially available DNA tests for cattle in the market and soon became a model example of the contribution of genome technology to animal breeding through marker-assisted selection (MAS).

QTL mapping and association studies have revealed that two genes are responsible for a high proportion of the genetic variation in beef tenderness. One of these genes codes for the large subunit of the protease μ-calpain, an enzyme involved in the process of tenderization of aged beef, and the other encodes calpastatin, which is a negative regulator of the calpains.

Association studies have shown that one of the most significant markers in *CAPN1* is the SNP *CAPN316*, located in exon 9 of the gene (AF252504.2:g.5709C>G, p. Ala316Gly; Page *et al.* 2002). Another common marker on the same gene, *CAPN4751*, is a polymorphism in intron 17 of the gene (AF248054.2:g.6545C>T; White *et al.* 2005).

In the case of *CAST*, there are two well-known markers that are associated with beef tenderness. The marker that will be referred to as *CAST1* is located in the 3′ untranslated region of the gene (AF159246.1:g.2959A>G; Barendse 2002), whereas the marker that will be referred to as *CAST2* is in intron 5 of the gene (AY008267.1:g.282C>G; Schenkel *et al.* 2006).

While several publications have addressed the association of polymorphisms in the *μ-calpain (CAPN1)* and *calpastatin (CAST)* genes with variation in beef tenderness (Casas *et al.* 2005, 2006; Morris *et al.* 2006; Corva *et al.* 2007), information about the effect of these markers on other

Address for correspondence

P. M. Corva, Department of Animal Science, College of Agricultural Sciences, University of Mar del Plata, CC 276, 7620 Balcarce, Argentina.
E-mail: pcorva@balcarce.inta.gov.ar

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economically important traits is still scarce. The existence of potential correlated unfavourable responses should be assessed before recommending the widespread use of these markers as a selection tool. In fact, experimental evidence suggests that the *calpains/calpastatin* system could have effects in the live animal that in turn may influence beef production, and therefore close consideration of associated unfavourable responses is warranted.

When allele frequencies of *CAPN316* are compared across breeds and lines within breeds, there is a trend towards a relatively lower frequency of the favourable allele in lines or breeds that have higher growth potential (Page *et al.* 2004; Morris *et al.* 2006; Corva *et al.* 2007; Van Eenennaam *et al.* 2007). Moreover, there is considerable overlap of QTL in the distal region of bovine chromosome 29 that spans *CAPN1* (http://www.animalgenome.org). These are QTLs regulating not only beef tenderness but also growth (weaning weight, carcass weight) and feed efficiency. While *CAPN1* is considered to be the best candidate for tenderness QTL, little is known about the genes underlying other QTLs in the region.

There are also some clues about the effect of markers in *CAPN1* and *CAST* in traits other than beef tenderness that have come from association studies. In the experiment of Cafe *et al.* (2010), Brahman steers with two favourable alleles at *CAPN4751* had lower body weight before entering the feedlot than their counterparts. No significant effects were detected for *CAPN316* or *CAST1*. Casas *et al.* (2005) reported a significant effect of *CAPN316* on hump height of Brahman steers, but not on hip height. Interestingly, in this case, the allele favouring tenderness was the one associated with lower hump height. This result was attributed not to *CAPN1* itself but to other tightly linked genes involved in growth regulation. In another experiment with Brahman cattle, carcasses from steers with favourable alleles in *CAST* and *CAPN4751* were leaner (Wolcott & Johnston 2009). Miquel *et al.* (2009) also reported a significant association of CAPN316 with daily weight gain and final weight of Brangus steers finished on pastures. In this study, the animals with unfavourable alleles at *CAPN1* grew faster. From all these results, it can be concluded that the current information is not conclusive; in fact, to some extent, it is contradictory. It must be recognized that the lack of consistency could be a consequence of the influence of genetic background effects, the existence of genotype x environment interactions and, in the case of linked genes, of linkage phase.

The availability of panels of molecular markers could have a profound impact in the selection for beef quality traits. The selection objective in beef cattle, however, usually includes several traits simultaneously. It is thus worthwhile to confirm that selection to improve tenderness has no adverse effects on other traits of economic importance.

In this work, the potential association of molecular markers for beef tenderness with growth traits was evaluated in Angus cattle from Argentina, one of the most widespread breeds in the country. To test this hypothesis, an experiment was designed that took advantage of the availability of quantitative and molecular information generated by the national sire evaluation.

At the request of the Argentine Angus Association, a private laboratory conducted an experiment to estimate allele frequencies of four well-known markers for beef tenderness. This was an initial step towards the implementation of MAS strategies to improve beef quality. A sample of 268 influential sires of this breed was genotyped for the markers *CAPN316*, *CAPN4751*, *CAPN1* and *CAST2*. For this study, the 2008 Edition of the Sire Summary, including expected progeny differences (EPDs) and genotype information, was provided by the Angus Association of Argentina, in Excel format. EPD information is summarized in Table 1.

Markers *CAPN316*, *CAPN4751*, *CAPN1* and *CAST2* were chosen by the Angus Association because they have been extensively evaluated, and alleles C, C, A and C respectively, are considered to be associated with higher beef tenderness. Frequencies of each favourable allele in the sample of Argentine Angus bulls, estimated for the present experiment with the ALLELE procedure of the SAS package (SAS, 1998), were 0.287 ± 0.021 for *CAPN316*, 0.765 ± 0.015 for *CAPN4751*, 0.788 ± 0.018 for *CAST1* and 0.91 ± 0.012 for *CAST2* respectively. These frequencies are consistent with previous estimations for Angus and other *Bos taurus* breeds (Page *et al.* 2004; Morris *et al.* 2006; Van Eenennaam *et al.* 2007).

The 2008 Edition of the Sire Summary included 1365 Black and Red Angus bulls, of which only 268 had marker information. In Argentina, Black and Red Angus are regarded as two varieties of a single breed. The identity of sires and dams of the bulls was available in the Sire Summary. To improve the connection between individuals with and without genotypes, the grandsires and granddams of bulls with genotypes were added to the database. The data were provided by the National Cattlemen Association ('Sociedad Rural Argentina', http://www.sra.org.ar/rrgg/), which is

Table 1 Number, mean, minimum, maximum and average accuracies (Accu.) of expected progeny differences in the Argentine Angus Sire Summary, 2008 Edition.

Trait	*n*	Mean	Min.	Max.	Accu.
BW (kg)	1365	0.16	−4.0	3.2	0.84
WWD (kg)	1361	5.10	−21.4	31.0	0.82
LW18 (kg)	1361	12.3	−34.0	51.5	0.71
REA (cm^2)	978	0.23	−7.8	10	0.66
BFT (mm)	978	0.10	−1.3	3.2	0.65
SC (cm)	1265	0.87	−2.2	3.1	0.78

BW, Birth weight; WWD, Weaning weight (Direct); LW18, Live weight recorded at 18 months of age; REA, Rib eye area; BFT, Back fat thickness; SC, Scrotal circumference.

responsible for the registration of all livestock species in the country. A segregation analysis was conducted to assign genotype probabilities for each marker (Kerr & Kinghorn 1996; http://www-personal.une.edu.au/~bkinghor/geneprob.htm). The segregation analysis assigns a genotype probability to non-genotyped individuals based on information from related animals in the population. Therefore, the 1365 bulls in the sire summary, including those with unknown genotype, could be included in the analyses. The same strategy has been recently implemented to include non-genotyped individuals in an animal model with marker effects (Baruch & Weller 2009).

Weighted least squares analysis was employed to study the effects of tenderness markers on EPDs for birth weight, weaning weight (direct), live weight at 18 month of age, rib eye area, backfat thickness and scrotal circumference. The model was $y_i = \beta_0 + \beta_1 x_i + e_i$, where y_i is the EPD of a sire for a given trait and x_i is the probability of having 0, 1 or 2 alleles favouring tenderness at a molecular marker. A similar study has been conducted for milk production traits and the *Osteopontin* gene in Holstein cattle (Leonard *et al.* 2005). Given the strong imbalance of allele frequencies for some of the markers, two-way interactions were not included in the models. Accuracies of EPDs were incorporated as weights in the model. A modified Bonferroni correction was used to take into account multiple comparisons (Schenkel *et al.* 2006). The statistical analyses were performed with the REG procedure of SAS (1998).

Results are presented in Table 2. There were significant effects of all markers for at least one trait. In all cases, with the exception of *CAST2* and weight at 18 months, the allele that favours tenderness is associated with lower EPD values. For example, given that C is the favourable allele for tenderness at *CAPN316*, the DEP for birth weight of sires with genotype CC at this locus would be 0.227 kg lower than the DEP of sires with genotype CG. Backfat thickness and scrotal circumference were not associated with any marker. *CAPN316* had significant effects on birth and weaning weight, suggesting that one or more genes in the distal region of BTA29 are involved in the regulation of early growth. Despite the significance of effects, the proportion of variance explained in each case was small and the highest value was for SNP316 and weaning weight (Table 2). This result is not surprising, given that a single marker was considered at a time. Independently of the influence of a marker on the variation of the trait, an unknown but probably very large number of genes are involved in growth regulation. In this experiment, two-marker interactions were not tested. Interactions between markers in *CAPN1* and *CAST* have been previously detected as significant in some experiments (Barendse *et al.* 2007), but not in others (Casas *et al.* 2006; Cafe *et al.* 2010).

The observed results can be the consequence of an effect of μ-calpain per se and/or the effect of tightly linked genes. Because there is also a significant effect of *CAST* markers, it seems that μ-calpain must be involved in some way in growth regulation. Interestingly, at least one QTL for carcass weight that is located close to *CAST* in BTA 7 has been reported recently (http://www.animalgenome.org), and there is experimental evidence of a role for the *calpains/calpastatin* system in muscle cell migration and differentiation at early stages of development (Dedieu *et al.* 2004; Moyen *et al.* 2004; Barnoy *et al.* 2005).

If the *calpains/calpastatin* system is involved in the regulation of growth, the strong emphasis on selection for growth rate and body size in many beef cattle breeds could explain, at least partially, the reported allele frequencies in markers such as *CAPN316*. In other words, because beef tenderness was not included in the selection objective, past selection could have worsened this trait despite its importance.

Any recommendations to improve beef tenderness using molecular markers in the *CAPN1* and *CAST* genes should take into account the fact that there could be a correlated response in growth traits. Therefore, a selection index or similar strategy is needed before these genes can be incorporated into selection schemes.

Table 2 Coefficients (β_1) and standard errors (SE) of the linear regression between the number of alleles favouring tenderness (0, 1, 2) at four molecular markers, and expected progeny differences (EPDs) of Argentine Angus Sires (Sire Summary 2008 Edition).

	CAPN316				*CAPN4751*				*CAST1*				*CAST2*			
	β_1	SE	*P*-value	R^2	β_1	SE	*P*-value	R^2	β_1	SE	*P*-value	R^2	β_1	SE	*P*-value	R^2
BW (kg)	−0.227	0.064	0.0004[1]	0.0091	−0.135	0.071	0.057	0.0027	−0.129	0.103	0.211	0.0011	0.061	0.071	0.398	0.0005
WWD (kg)	−1.767	0.423	0.0001[1]	0.0126	−1.111	0.469	0.018	0.0041	−1.637	0.682	0.017	0.0042	0.724	0.473	0.126	0.0017
LW18 (kg)	−2.101	0.753	0.005[2]	0.0057	−1.866	0.832	0.025	0.0037	−3.834	1.202	0.002[1]	0.0074	2.458	0.835	0.003[2]	0.0063
REA (cm^2)	0.052	0.154	0.735	0.0001	−0.501	0.169	0.003[2]	0.0089	0.316	0.247	0.200	0.0017	0.278	0.171	0.104	0.0027
BFT (mm)	0.063	0.036	0.079	0.0032	−0.039	0.039	0.316	0.0010	−0.065	0.057	0.258	0.0013	−0.053	0.040	0.180	0.0018
SC (cm)	−0.059	0.042	0.161	0.0016	0.022	0.047	0.632	0.0002	0.007	0.68	0.916	0.0000	0.046	0.047	0.334	0.0007

[1]Highly Significant effect ($P < 0.01$) after modified Bonferroni correction for experiment-wise multiple tests.
[2]Significant effect ($P < 0.05$) after modified Bonferroni correction for experiment-wise multiple tests.

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