ASSOCIATION OF THE EXON 9 SINGLE-NUCLEOTIDE POLYMORPHISM OF CAPN1 WITH BEEF TENDERNESS

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SUMMARY

Initial data analysis from both the University of Adelaide's Davies Cattle Gene Mapping and the New Zealand AgResearch Cattle Gene Mapping Projects showed a tenderness quantitative trait locus (QTL) on BTA29. Based on its function and location, the gene for micromolar calcium-activated neutral protease or calpain gene (CAPN1) was considered to be a strong positional candidate for the observed QTL effects. The objective of this study was to assess the association of a previously reported single nucleotide polymorphism (SNP) in Exon 9 of CAPN1 with the tenderness of *M. longissimus dorsi* (LD) and *M. semitendinosus* (ST) muscles in *Bos taurus*. The SNP (base 5709) causes the amino acid substitution of alanine for glycine^{316} in the μ-calpain enzyme. Results demonstrated that the Exon 9 SNP was significantly associated with tenderness of both ST and LD muscles ($P<0.01$). Animals with the GG genotype showed higher ($P<0.01$) shear force values compared with CC genotypes (15% and 11% on day 1 and 14% and 11% on day 26 in LD and ST, respectively). The paternal allele encoding glycine at position 316 was associated with decreased meat tenderness relative to the allele encoding alanine at position 316. The equivalent maternal allele for CAPN1 Exon 9 was significantly associated with decreased tenderness of LD at four cook times and ST at day 1.

Keywords: beef cattle, meat tenderness, quantitative trait loci, CAPN1.

INTRODUCTION

Tenderness of meat, which has a significant impact on consumer satisfaction, is a current concern in the beef industry. However, genetic improvement of meat tenderness as well as other aspects of carcass and meat quality by classical phenotypic selection is often limited because trait measurements can only be obtained on slaughtered animals. Detection of genes affecting these traits allows characterization of the genetic potential of living animals at a very young age (potentially as young as an embryo).

Initial analyses of both New Zealand AgResearch and the University of Adelaide's Davies cattle gene mapping projects revealed a significant QTL for tenderness on BTA29 close to the mapped position of the micromolar calcium-activated neutral protease or calpain gene (CAPN1) (Page *et al*. 2002). The CAPN1 gene encodes the larger part of μ-calpain, a protease that appears to be the primary enzyme in the *post mortem* tenderization process (Koohmaraie, 1996). Smith *et al.* (2000) mapped CAPN1 to the telomeric end of BTA29 and Page *et al*. (2002) showed that SNPs in CAPN1 are markers for meat tenderness variation. The objective of this study was to evaluate the association of a SNP in the CAPN1 gene with tenderness of the *M. longissimus dorsi* (LD) and *M. semitendinosus*

(ST) muscles. The SNP is a cytidine/guanosine (**C/G**) polymorphism in exon 9 of the gene (base 5709), which results in the amino acid substitution of glycine^{316} with alanine.

MATERIAL AND METHODS

Animals. A three-generation resource population, the University of Adelaide's Davies Cattle Gene Mapping herd in Australia was developed using two phenotypically extreme divergent breeds, Jersey (J) and Limousin (L). Details on the family structure and management of the animals are given in the associated paper (Esmailizadeh Koshkoih, *et al*. 2005)**.**

Tenderness measurements. Meat tenderness was measured on backcross progeny (n=356) from 3 families. The animals were killed in a commercial plant and carcasses were electrically stimulated with a low voltage (peak 45V, 200mA) rectal-nostril stimulator for 40 seconds within five minutes of sticking. Following standard line processing, the carcasses were weighed, split and stored in a chiller (0-4°C) overnight. The left half-carcass from each animal was boned out and samples collected from the eye round (*M. semitendinosus*) and striploin (*M. longissimus dorsi*). Samples of 2.5 cm steaks were vacuum packed, randomly assigned to different ageing treatment groups (1, 5, 12, 26 days) at 2°C, and frozen (-20°C) after the completion of the ageing treatment. Before the tenderness measurements, the steaks were thawed overnight at 2°C and trimmed to 80-100g samples. pH was recorded prior to cooking using a WP-80 pH, mV, Temp-meter. Samples were cooked at 70°C for 40min in a water bath and cooled in running water. After storage overnight in the chiller, rectangular strips (15.0 x 6.6mm) were cut parallel to the fibers and Warner-Bratzler shear force measurements were performed on the Lloyd pressure tester according to Bouton *et al* (1971).

SNPs genotyping and haplotype construction. All backcross progeny were genotyped for the Exon 9 SNP of CAPN1 that changes the protein sequence by the amino acid substitution of alanine for glycine (G^{316}/A^{316}) in Domain II of the protein. Only one of the sires (368) was heterozygous for the Exon 9 SNP in the Australian gene mapping herd. The haplotypes were identified based on flanking micro-satellite markers inherited by sire 368 from the Jersey grand-dam and Limousin grand-sire. Haplotypes were inferred in progeny homozygous for the SNP and by comparing flanking markers used in QTL analysis. The sire-derived SNP allele in all animals was defined by haplotype. Dam alleles were predicted by subtraction of the known sire haplotype from the genotypes of the progeny.

Statistical analysis. Association analyses were carried out to evaluate the relationship between the different SNP genotypes and Warner-Bratzler values. The statistical model used included fixed effects of SNP genotype (three classes), breed of dam (Jersey or Limousin), cohort (combination of sex and year), sire and all possible two-way interactions among the effects. The residual effect was the random term in the model. All interactions that were not significant were dropped from the final model. An alternative model was tested in which, the sire-derived allele (two levels) and dam-derived allele (two levels) were fitted to see whether the same effect was present in the dam population, as an independent test. Additive genetic effects were computed as the difference between the solutions of the estimate for the effect of the two homozygous genotypes (GG-CC). Dominance deviation was computed by subtracting the average of the estimates for the two homozygous genotypes from that of the heterozygote genotype (GC- [(GG+CC)/2]).

RESULTS AND DISCUSSION

The mean shear forces were very low (Table 1) and showed little change over time with a CV of around 25% for the LD and 17% for ST at each time point. The effects of the Exon 9 CAPN1 SNP genotype on tenderness showed that animals with the genotype CC had more tender meat measured in both the LD and ST muscles. Animals with the GG genotype showed higher (P<0.01) shear force values compared with CC genotypes (15% and 11% on day 1 and 14% and 11% on day 26 during aging in both the LD and ST muscles, respectively). The G allele was significantly associated with increased shear force and showed a significant additive effect in both LD and ST muscles at four aging times. Aging time by genotype interaction was not significant. Although there was a significant dominance deviation of the G allele in the ST muscle at days 5 and 26 after slaughter, the dominance effect on the LD muscle was not significant (Table 2).

Table 1. Association of SNP genotypes in Exon 9 CAPN1 (least square means ± SE) with tenderness of *M. semitendinosus* (ST) and *M. longissimus dorsi* (LD) muscles measured as Warner-Bratzler shear force (kg) at four cook times (days after slaughter)

Muscle	Time (days)	Exon 9 CAPN1 genotype			P-value[a]
LD		GG	GC	CC	
# animals	328	165	141	22	
	1	5.06±0.09	4.52±0.10	4.39±0.27	***
	5	4.55±0.07	4.15±0.08	4.05±0.20	***
	12	4.26±0.07	3.91±0.08	3.77±0.21	**
	26	3.97±0.06	3.69±0.07	3.45±0.19	**
Mean		4.45±0.07	4.07±0.07	3.89±0.19	***
ST					
# animals	325	163	140	22	
	1	5.29±0.06	5.04±0.06	4.77±0.16	**
	5	4.96±0.05	4.92±0.06	4.46±0.15	**
	12	4.85±0.05	4.72±0.05	4.33±0.14	**
	26	4.56±0.05	4.50±0.05	4.11±0.13	**
Mean		4.91±0.04	4.79±0.04	4.42±0.12	***

[a]Testing mean differences of genotypes where ** = P< 0.01, *** = P<0.001

The sire dam alleles (when analysed simultaneously with the dam haplotype as a fixed effect with regard to shear force) indicated that the G allele (Limousin derived) was associated with tougher meat at the first two cook times for the LD muscle and the last two cook times for the ST muscle. The dam contributions revealed a significant effect of the same dam allele on meat tenderness, providing strong evidence for the hypothesis that this SNP is in linkage disequilibrium with the functional allele and it could be the functional allele in the quantitative trait nucleotide (QTN).

Confirming the report of Page *et al.* (2002) regarding the association of the Exon 9 CAPN1 SNP with meat tenderness, the results herein present further evidence that this DNA variant may be the causal SNP for the tenderness QTL on BTA29 now observed in several studies (Page *et al*. 2002; Casas *et*

al. 2000). Moreover, the SNP is associated with tenderness in two muscles of different types, the oxidative *M. longissimus dorsi* and the glycolytic *M. semitendinosus*.

There is considerable interest in the application of molecular technologies in the form of specific DNA markers that are associated with traits that are difficult or expensive to measure in order to promote efficient selection of animals (Spelman and Bovenhuis, 1998). Meat tenderness is an example of such a trait and if the SNP in Exon 9 of CAPN1 can reliable predict meat tenderness in all muscles in commercial beef cattle populations, it will provide a valuable tool to improve meat tenderness via marker-assisted selection.

Table 2. Shear force differences of sire and dam alleles (defined as G-C), additive effect (*a*) and dominance deviation (*d*) of the Exon 9 CAPN1 SNP

Muscle	Time (days)	Sire (G-C)	P	Dam (G-C)	P	*a* ± S.E.	P	*d* ± S.E.	P
LD	1	0.59±0.22	**	0.39±0.14	**	0.33±0.07	*	-0.21±0.16	ns
	5	0.53±0.16	**	0.25±0.11	*	0.25±0.06	*	-0.14±0.12	ns
	12	0.42±0.17	*	0.26±0.11	*	0.25±0.06	*	-0.10±0.13	ns
	26	0.32±0.15	*	0.24±0.10	*	0.26±0.05	**	-0.01±0.11	ns
ST	1	0.26±0.13	+	0.26±0.09	**	0.27±0.04	**	0.01±0.10	ns
	5	0.29±0.12	*	0.06±0.08	Ns	0.25±0.04	**	0.22±0.09	*
	12	0.53±0.11	***	0.04±0.07	Ns	0.26±0.04	***	0.13±0.09	ns
	26	0.31±0.11	**	0.07±0.07	Ns	0.22±0.03	**	0.17±0.08	*

[a] ns = non-significant, * = P <0.05, ** = P< 0.01, *** = P<0.001

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