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A single-nucleotide polymorphism on Calpain-1 is associated with meat tenderness in cattle

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ABSTRACT

Variability in tenderness remains a major concern to the meat industry, and our objective was to identify genetic effects on beef meat tenderness, in animals treated alike, and under similar *post-mortem* processing conditions. A quantitative trait locus (QTL) for beef tenderness has been located on the same region of chromosome 29 in a United States Department of Agriculture (USDA) study and in our research. Micro-calpain, or calcium-activated neutral protease (CAPN1), has been identified as a candidate gene. The calpain/calpastatin system is involved in the change of tenderness with time, by regulating *post-mortem* proteolysis. The CAPN1 gene was sequenced by USDA workers who identified single-nucleotide polymorphisms (SNPs) in two exons. These results were followed up as part of the AgResearch Jersey-Limousin Beef QTL trial, where steak samples were obtained from the *longissimus dorsi* of 416 animals slaughtered at about two years of age. The segregating USDA and NZ families were both heterozygous for one of these SNPs, on exon 9, and this region was predicted to alter the protein sequence by the substitution of alanine for glycine in Domain II. Both families were genotyped for these two SNPs, as well as for six intronic polymorphisms, to define haplotypes. Analysis of tenderness measurements in the NZ data (n=81 animals) showed a difference between paternal CAPN1 haplotypes, with the SNP encoding alanine at amino acid number 316 being associated with more tender meat (decreased shear force) relative to the SNP encoding glycine ($P < 0.00001$). The association of the maternal haplotypes with meat tenderness phenotypes ($P < 0.01$) was also consistent with the hypothesis of CAPN1 as the gene underlying the QTL effect. The sire and dam effects together accounted for over 30% of the residual variance in tenderness. Our results show that a single SNP test in the laboratory can distinguish genetically tender and genetically tough animals at any age.

Keywords: cattle; meat; tenderness; QTL; micro-calpain; SNP.

INTRODUCTION

A collaborative genetic marker project between AgResearch and Adelaide University was established in 1995 to search for quantitative trait loci (QTL) affecting beef carcass composition and meat quality. The present report shows that meat tenderness in this project is associated with a single-nucleotide polymorphism (SNP) on a candidate gene under a QTL on chromosome 29 (BTA29). Research was also carried out in collaboration with the United States Department of Agriculture (USDA) who had also been searching for tenderness QTL (Page *et a*l., 2002).

MATERIALS AND METHODS

Design

A double back-cross genetic marker design was established in 1995, using bulls that were first crosses between two *Bos taurus* breeds known to be widely different in a number of performance traits (Cundiff *et al.*, 1986). The two breeds were Jersey (J) and Limousin (L). Three pairs of half-brothers were generated as first crosses (X), and one of each pair was used for mating in each country over both J and L cows to produce back-cross cattle. In New Zealand, 261 experimental back-cross calves (162 XJ and 99 XL) were born in spring 1996, and another 155 were born in spring 1997 (102 XJ and 53 XL). Offspring were slaughtered at ages ranging from 22 to 28 months at the Ruakura Abattoir. In Australia, about 400 experimental back-cross calves were born over the three years 1996-98. This paper reports meat tenderness data from cattle in the New Zealand herd.

Slaughter and muscle measurements

Animals from the two birth years were pre-allocated to slaughter groups in the springs of 1998 and 1999 over 18 and 10 kill days, respectively (one slaughter group per week, containing approximately 15 same-sex animals). Pre-allocation was based on breed of calf, and sire, and balanced as far as possible within breed for live weight before the first slaughter day. Each animal was stunned by captive bolt and then slaughtered, with no electrical stimulation applied on the chain. After splitting the carcass into two sides, the right striploin was removed for processing.

Initial pH was recorded within about 30 minutes of slaughter and then monitored at intervals for about 24 hours until *rigor mortis* ($pH < 5.5$), while the striploin was held in a controlled-temperature cabinet at 15°C. Five steak portions were then cut from the striploin for cooking and shear-force measurements. The first steak was processed on reaching *rigor mortis*, and the four other steaks were cooked at intervals (at approximately 1.3, 2.0. 2.3 and 4.0 days *post mortem*), after continued storage at 15°C. For cooking, each steak was placed inside a plastic cooking bag, heated in a boiling water-bath to an internal temperature of 75°C, then removed and cooled rapidly in ice to an internal temperature of 2°C. Shear-force measurements were then recorded using a MIRINZ tenderometer (Fraserhurst & MacFarlane, 1983), taking the average from measurements of ten 1cm x 1cm cores, aligned with the fibres running longitudinally along the core. The measures of tenderness on the five steak samples were referred to as COOK 1-5.

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For animals slaughtered in 1998, micro-calpain (mu-CALP), milli-calpain and calpastatin concentrations were measured from fresh 5g samples collected from the right striploin, as described by Wheeler and Koohmaraie (1991), with modifications (Sainz *et al*., 1992).

Statistical analyses

The half-life for the decline in shear force (SHLF) was calculated by fitting an exponential decay function through the shear-force results from COOK 1-5 (Dransfield *et al*., 1980-81), for each animal separately. The average shear force of COOK 1-5 was also calculated (SAVG).

Results for the traits defined above were analysed using JMP (SAS Institute Inc., 1995), fitting relevant fixed effects for breed group x birth rank (n=3 classes, with one for XJ calves and two for XL calves to allow for singles and twins born as a result of embryo transplantation), sire of calf, and slaughter group, which effectively adjusted for sex and year as well. Residual standard deviations (RSD) were obtained for each trait and used in a SAS procedure (SAS Institute Inc., 1999) to search for QTL across the 29 bovine autosomes.

Sequencing

The mu-CALP gene, (calcium-activated neutral protease, CAPN1), was identified as a candidate for tenderness under the QTL on BTA29 in a USDA population (Smith *et al*., 2000). To identify variations in the CAPN1 coding sequence that might explain the QTL effect, the complete gene was sequenced by USDA, apart from 100 kilobases (kb) of intron 10. In total, more than 28 kb of sequence containing the entire coding region (22 exons) was determined. This procedure identified five exonic SNPs of which two were predicted to cause amino acid variation in the mu-CALP protein.

A selection of eight SNPs that were informative in sire family 417, including the two that were predicted to cause amino acid changes and six intronic SNPs, were used to generate haplotypes. Progeny inheriting a complete series of homozygous SNPs were used to infer the two sire haplotypes and then sire-derived haplotypes were assigned to each progeny where possible. Dam haplotypes were inferred by subtraction of the known sire haplotype from the genotype of the calves. The sire and dam haplotypes were then fitted as fixed effects in an analysis of the residual shear forces.

RESULTS

Defining haplotypes

Table 1 shows the overall means and RSDs for the traits described. A significant QTL on BTA29 was detected for COOK 2, 3, 4, SAVG shear force and SHLF in one family (#417). At this stage USDA had found a QTL for tenderness in the same region of BTA29 and were sequencing a candidate gene, CAPN1 in order to identify SNPs. Of the two SNPs that coded for amino acid changes in the NZ pedigrees, one was heterozygous for two families (394 and 417) and the other (Glycine (G) or Alanine (A) at amino acid number 316) was heterozygous for family 417 only.

TABLE 1: Overall means, and residual standard deviations (RSD) for beef meat tenderness traits, after adjusting for breed group, sire and slaughter group.

Trait		Units	Mean	RSD
Tenderness				
Shear Force1	COOK1	kgF	14.60	2.21
Shear Force 2	COOK2	kgF	10.89	2.28
Shear Force 3	COOK3	kgF	7.45	1.56
Shear Force 4	COOK4	kgF	6.80	1.23
Shear Force 5	COOK5	kgF	5.84	0.94
Average shear force	SAVG	kgF	9.11	1.35
Half-life of shear-force change	SHLF	hr	12.42	6.54
Calpain system				
micro-calpain	mu-CALP	i.u./g	0.94	0.16

Of the 114 progeny from family 417 genotyped for eight SNPs, we were able to determine sire-derived haplotypes for 81 calves and, therefore, the allele inherited from the sire for these calves. Allele 1 was arbitrarily defined as G and allele 2 as A for the SNP of interest. Alleles 1 and 2 were identified as being inherited from the Limousin and Jersey parent of the sire, respectively. An analysis of the shear-force data fitting this SNP showed that the Limousin allele (G) was associated with increased toughness in this population. This was consistent with the original microsatellite-based QTL analysis (as would be expected for any marker closely linked to the QTL). It also matched the USDA data where the G allele derived from the Piedmontese parent was associated with increased shear force; the A allele came from the Angus parent.

Dam-derived alleles were inferred for 76 calves. The frequency of the A allele (associated with tenderness in the sire) was 47% overall, and identical within the two dam breeds (J and L). For the 76 animals whose parental contributions could be determined, the two types of heterozygotes, classified according to parental origin, were very similar in mean tenderness, and fell almost half-way between the two homozygote classes. We therefore believe it is a reasonable assumption to ignore the source of the allele, and to analyse the genotype with three levels (AA, AG and GG) instead of four. This allowed the inclusion of a further 32 progeny that were heterozygous for all the SNPs used for the haplotypes.

Sire and dam effects

Table 2 summarises effects on COOK 2, 3 and 4 associated with each of the haplotypes inherited from the sire. There was no significant effect on COOK1 and a lesser level of significance on COOK5 ($P = 0.02$), suggesting that the gene has little effect on initial tenderness and a minor effect on ultimate tenderness but has a major effect on the rate of increase of tenderness with time. This is reflected in the significant effect of the sire haplotype on SHLF, another measure of the intermediate shear forces. The effects of the allele inherited from the dam and from the sire were fitted together, in order to confirm that the effect was the same if inherited from either parent. There was no significant interaction between the sire- and dam-inherited alleles.

The estimates for progeny genotype with three levels (ignoring parental source in the heterozygote) are also presented in Table 2. The sire and dam effects accounted

TABLE 2: Effects of sire and dam haplotype on the least-square mean ± standard error, and probability values for meat tenderness at cooking times 2, 3 and 4 (1.3, 2.0 and 2.3 days *post mortem*).

		Shear force (kg)		
Alleles	**Number of progeny**	**COOK2**	**COOK3**	**COOK4**
Sire				
Haplotype 1 (ex Limousin)	31	12.01±0.36	8.55±0.24	7.68±0.19
Haplotype 2 (ex Jersey)	50	9.75±0.27	6.86±0.18	6.33±0.15
Difference		2.26±0.45	1.69±0.30	1.35±0.24
Effect (SD[a] units)		0.99	1.08	1.10
Probability value		3×10^{-6}	2×10^{-7}	3×10^{-7}
Dam[b]				
G (glycine)	40	11.51±0.30	8.02±0.20	7.37±0.16
A (alanine)	36	10.12±0.35	7.29±0.23	6.58±0.19
Difference		1.38±0.48	0.73±0.32	0.79±0.26
Effect (SD[a] units)		0.61	0.47	0.64
Probability value		0.0049	0.026	0.003
Progeny				
GG	24	12.46±0.39	8.96±0.27	7.96±0.20
AG	54	10.83±0.26	7.41±0.18	6.87±0.14
AA	30	9.28±0.34	6.68±0.23	6.11±0.18
Probability value		1×10^{-7}	2×10^{-8}	3×10^{-9}

[a] SD = standard deviation
[b] After fitting the sire effect

for over 30% of the residual variance in tenderness. Figure 1 presents the results for SHLF and the concentration of mu-CALP for the three progeny genotypes showing a decrease in SHLF relative to an increase in mu-CALP.

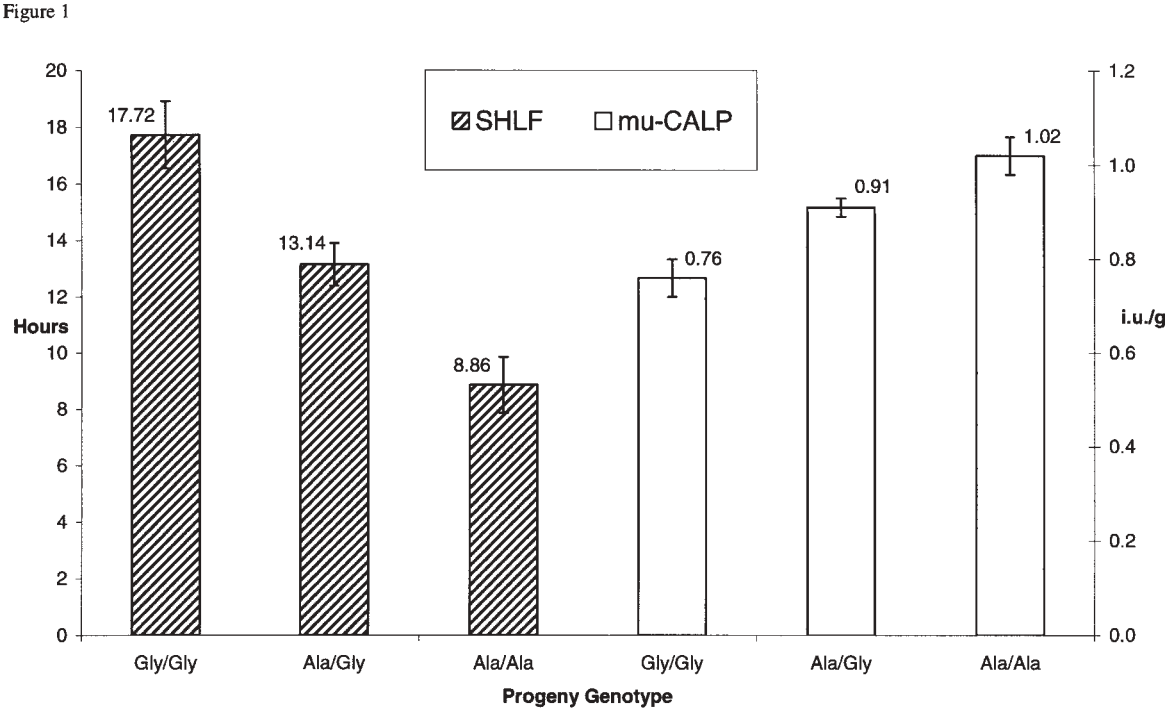
DISCUSSION

The NZ results provide additional evidence to the USDA results that variation in the CAPN1 gene is associated with meat tenderness. The fact that sire 417 with a QTL for tenderness was the only sire that was heterozygous for the G/A SNP is consistent with this SNP being the causative mutation. The association of the SNP with tenderness was the same if derived from sires or dams.

Although our resource population was from a back-cross design, the Limousin and Jersey sires originated in Australia, whilst the Limousin and Jersey dams were relatively distinct as they originated in New Zealand.

The fact that the SNP coded for an effect with the same sign in Limousins and Jerseys in our study, and also in Piedmontese and Angus in the USDA study, gives additional weight to the theory that the SNP probably

FIGURE 1: Effect of the glycine (Gly)/alanine (Ala) SNP on shear-force half-life (SHLF) and micro-calpain (mu-CALP) concentration, measured in *longissimus dorsi* samples from back-cross cattle.



originated many generations ago before the breeds diverged, and there is a neutral effect on fitness.

The effect of the heterozygote was roughly intermediate between that of the two homozygotes, but slightly closer to AA (tender genotype) in the case of COOK 3 and 4. This is important because animals carrying just one A allele will thus be improved for SHLF and SAVG by at least half of the difference between GG and AA.

It was also notable that our genotypic results for the commercial measure, tenderness, were consistent with those for concentration of the mu-CALP protein itself, as was reported at the phenotypic level, for example, by Zamora *et al*. (1996) who found a negative relationship between shear force and mu-CALP. A definitive test of our SNP being part of the causative mutation for differences in toughness/tenderness through differences in CAPN1 would however need a measure of enzymic activity per unit of enzyme present in CAPN1 purified from the two homozygote types.

A single SNP test in the laboratory is now possible to determine the genotype of young growing stock, or of potential sires, for tenderness (at least for the breed types already tested). The SNP test could be done at any age, so that animals could be drafted for different growing-out regimes or for different markets, from a young age. In Australia, Genestar (2003) also have a DNA test for beef meat tenderness, and this uses variation in the CAPN1 inhibitor, calpastatin.

Analysis of shear-force values in the NZ data (n=81 animals) showed a difference between paternal CAPN1 haplotypes, with the SNP encoding alanine at position 316 being associated with more tender meat (decreased shear force) relative to glycine ($P < 0.00001$). The association of the maternal alleles with meat tenderness phenotypes ($P < 0.01$) was also consistent with the hypothesis of CAPN1 as the gene underlying the QTL effect, as was the finding of the same haplotype and phase in all four breeds. The sire and dam effects accounted for over 30% of the residual variance in tenderness. Our results show that a single SNP test in the laboratory is now available to distinguish genetically tender and genetically tough animals at any age.

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