CALPASTATIN AS A CANDIDATE GENE FOR SHEEP MEAT TENDERNESS

B.R. Palmer, N. Roberts, M.P. Kent, M.A. Ilian, C. Le Couteur, J. Morton, J.G.H. Hickford, R. Bickerstaffe
Molecular Biotechnology, Animal and Veterinary Sciences, PO Box 84, Lincoln University, Canterbury, New Zealand.

Keywords: ovine calpastatin; genetics; tenderness; polymorphic variation

Introduction

The discipline of the molecular genetics of production animals is developing rapidly and has enormous potential in helping to improve quality. Much of the activity in domestic animal genetics involves mapping quantitative trait loci (QTLs) using molecular approaches that entire animal genomes, followed by the prediction of regions likely to contain QTLs for specific phenotypes. Such "whole genome" approa are particularly useful when there is no obvious candidate genes for a specific trait (Crawford *et al.*, 1997, Montgomery *et al.*, 19 However there are phenotypes where, because of previous biochemical and physiological investigation or theoretical extrapolation from o species, at least one candidate gene can confidently be predicted. In these cases a candidate gene approach to understanding the genetics particular animal quality trait can be justified, without prior genome scanning for QTLs. The calpain-calpastatin system (CCS) of calc dependent neutral proteases (μ- and m-calpains) and their specific inhibitor, calpastatin, have emerged as having the primary role in the p mortem myofibrillar degradation responsible for meat tenderisation (Koohmaraie *et al.*, 1995; Dransfield , 1994). In beef cattle the calpas gene (CAST) has been implicated as an important gene influencing ultimate meat tenderness (Shackelford *et al.*, 1994). As yet, polymo variation in the bovine gene (Koohmaraie *et al.*, 1995) has not been found to be associated with differences in meat tenderness (Lonergan *et a* 1995). It is hypothesised that variation in calpastatin levels in post-mortem ovine muscles is, at least, due in part to variation in the CAST loc

Objectives

In this study we have chosen CAST as a candidate gene for sheep meat tenderness because of the above evidence in cattle and data implic a similar role for the CCS in the tenderisation of meat in aging ovine carcasses (Morton *et al.*, 1997). The polymorphic variation found in ovine CAST locus has been tested for associations with differences in meat quality characteristics.

Methods

Purebred sheep from three breeds (Dorset Down [DD], Corriedale and Coopworth) and one mixed breed flock ("Ruakura") were bled t provide DNA for PCR-SSCP genotyping of CAST(Roberts *et al.*, 1996). Amplimer DNA from homozygote ***aa, ac*** and ***bb*** animals we excised from 1% LMP agarose bands and purified using a Promega Wizard™ PCR Preps kit and subsequent ethanol precipitation. The DN was sequenced by dideoxy-dye chain-termination chemistry using the PCR primers calpsu and calpsd. Termination products were analysed o an Applied Biosystems Automated Sequencer at the Centre for Gene Technology, School of Biological Sciences, University of Aucklan PCR products from a sheep of *ac* genotype were cloned into the pCR2000 vector (Invitrogen TAII Cloning System, Invitrogen Corporatio San Diego, USA). Representative clones giving SSCP banding patterns indicative of alleles *a* and *c* were purified with a Qiagen miniprep k and sequenced with both forward and reverse universal primers. Slaughter trials used the procedures of Morton *et al.*, (1997).

Results and Discussion

Polymorphic Variation in CAST

Polymorphic variation in the exon 1C/1D region of ovine CAST was assessed using PCR-SSCP genotyping. Three alleles (*a*, *b* and *c*) hav been detected (Roberts *et al.*,1996). The relative frequencies of CAST alleles in four different flocks are shown in Table 1. Allele *a* i predominant in these flocks with varying frequencies for ***b*** and ***c***. All possible combinations of the three alleles, with the exception of homozygotes have been found in the DD flock, the most intensely studied flock.

Nucleotide sequence analysis of animals (n = 3) homozygous for alleles ***a*** and ***b*** revealed the amplimers were 612 bp in length and six singl base differences between the ***a*** and ***b*** allele sequences. All the differences occur within the intron between exons 1C and 1D. Five of the six sequence differences lead to changes to restriction endonuclease recognition sites (Fig. 1). None of the sequence differences appear to alte sequence features that would affect the efficiency of intron splicing, but they may affect message editing, mRNA stability or other mRNA processing. Preliminary sequencing of amplimers from ***ac*** CAST heterozygotes (n=2) and sequencing of plasmid clones of amplimers from one ***ac*** heterozygote suggest the difference between the ***a*** and ***c*** alleles is a single T insertion (sense strand) in a run of four T nucleotides (positions 514- 517) in the 3' polypyrimidic region of the intron (Fig 1). This region is important in intron splicing (Darnell *et al.*, 1986).

CAST polymorphism and meat quality

Genotyped DD sheep and DD x Coopworth lambs were slaughtered in two separate trials to determine if an association exists between the CAST alleles and meat quality characteristics. The results are summarised in Table 2. The rarity of homozygous ***bb*** sheep and the lack of ex homozygotes meant that only comparisons between ***aa***, ***ab*** and ***ac*** genotypes were possible. In trial I (purebred DD only) yearling sheep of genotype ***ab*** for CAST had mean ultimate meat pH values (measured in LD muscle at 24 h post-mortem) significantly higher than animals genotyped ***aa*** or ***ac*** ($p<0.01$). Secondly, ewes genotyped ***ac*** produced fillets with significantly higher shear force measurements than ***aa*** or ***ab*** animals ($p <0.05$). In trial I the differences in shear force were paralleled by changes in components of the CCS. With many measured parameters in trial I, rams had to be excluded to achieve significance when plotting parameters against genotype, suggesting an over-riding sex effect independent of CAST genotype. The exception was LD calpastatin activity at 0 time ($p = 0.04$) in trial I and preslaughter liveweight

carcass weight and LD m-calpain (0 time) in trial II (DD x Coopworth lambs) when genotype was the variable parameter. A similar but less significant sex effect was observed in Trial II. Smaller differences in ewe LD and *P. major* tenderness (not shown) in Trial II may be due the animals being younger than those used in Trial I.

References

- Crawford, A.M. et al. (1997). Proc. NZ Soc. Anim. Prod. **57**: *submitted*
- Darnell, J., Lodish, H. & Baltimore, D. (1986). *Molecular Cell Biology*. Scientific American , New York.
- Dransfield, E. (1994). Meat Sci. **36:** 105-121.
- Koohmaraie M., Killefer J., Bishop M.D., Shackelford S.D. Wheeler T.L. & Arbona J.R.. (1995). In: *Expression of tissue proteinases and regulation of protein degradation as related to meat quality* (ed. by A. Ouali, D.I. Demeyer & F.J.M. Smulders)pp 395-412. ECCEAMST, Utrecht, The Netherlands.
- Lonergan, S.M., Ernst, C.W., Bishop, M.D., Calkins, C.R. & Koohmaraie, M. (1995). J. Anim. Sci. **73:** 3608-3612.
- Montgomery, G.W., Lord, E.A. & Lumsden, J.M. (1997). Proc. NZ Soc. Anim. Prod. **57**: *submitted*
- Morton, J.D., Bickerstaffe, R., Le Couteur, C.E. & Keeley, G.M. (1997). ICOMST **43**: *in press*.
- Shackelford, S.D., Koohmaraie, M., Cundiff, L.V., Gregory, K.E., Rohrer, G.A., & Savell, J.W. (1994). J. Anim. Sci. **72:** 857-863.
- Roberts, N., Palmer, B.R; Hickford, J.G.H. & Bickerstaffe, R. 1996. Anim. Genet. **27:** 211.

Table 1: Calpastatin exon 1C/1D allele frequency in 4 flocks of sheep.

	Calpastatin exon 1C/1D allele frequency (per chromosome)			
Allele	Dorset Down (n=27)*	Corriedale (n=30)*	Coopworth (n=120)*	"Ruakura" (n=48)*
a	0.69	0.46	0.696	0.49
b	0.18	0.27	0.004	0.10
c	0.13	0.27	0.300	0.41

*number of individual sheep (unrelated ewes for Dorset Down, unrelated rams for Corriedale, related ewes for Coopworth and unknown relatedness for Ruakura) screened.

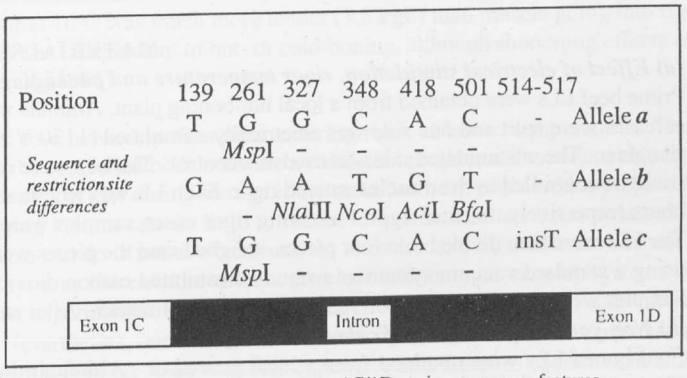

Fig.1. Ovine calpastatin gene exon 1C/1D region sequence features. Nucleotides shown differ between alleles. Restriction sites shown exist or are predicted to exist in the allele above. Amplimer length is 612 bp.

Table 2. Summary and interpretation of the meat quality characteristics data set from two slaughter trials.

Meat Quality Characteristic	Putative Association with Genotype	Statistical Significance*	Comments
A Trial I - Purebred Dorset Down Yearling Sheep[a]			
Tenderness/pH			
Psoas major shear force in ewes (Fillet tenderness)	*aa<ab<ac*	p<0.05	Fillet from *ac* animals significantly tougher than that from other genotypes
LD shear force in ewes (Back strap or chop tenderness)	*aa<ab<ac*	p<0.17	Fillet from *ac* animals marginly tougher than that from other genotypes
LD pH (24 h)	*aa < ab*	p<0.01	LD pH (24 h) is higher in *ab* animals than aa Highly significant Potentially commercially valuable
Enzymes			
LD calpastatin activity at 0 time	*aa <ac*	p=0.04	Possible explanation for both rams and ewes tougher meat
LD calpastatin activity at 0 time	*aa <ab <ac*	p=0.04	Possible explanation for *ac* ewes tougher meat
LD μ-calpain at 1 h	*aa >ab >ac*	p=0.03	Effect on calpastatin to μ-calpain ratio.
B Trial I I - Dorset Down x Coopworth Halfbred Lambs (mean age 141 days)[a]			
Animal weight			
Preslaughter liveweight	*aa < ac*	p=0.03	Independent of sire effect
Carcass weight	*aa < ac*	p=0.02	Independent of sire effect
Enzymes/pH			
LD m-calpain activity at 0 time	*aa > ac*	p=0.01	May explain for differences in LD tenderness
LD pH (24 h)	*aa < ab*	p=0.14	Similar phenomenon as seen in Trial I?
LD μ-calpain/calpastatin ratio at 0 time	*aa < ac*	p=0.20	May explain for differences in LD tenderness

* Statistics in trial I produced by ANOVA analysis using MINITAB 9.2 and in Trial II by 2-sample t-test using MINITAB 9.2 (State College , PA).

[a] Trail I design - 11 *aa*, 13 *ab* and 5 *ac* yearling purebred DD sheep were slaughtered slaughtered commercially and meat samples taken and analysed as described by Morton *et al.*, (1997). Parameters measured were carcass weight, LD 24 h tenderness, *Psoas major* 24 h tenderness, LD calpastatin, μ- and m-calpain at 0 and 12 hours and 90 min and 24 hr pH. Trial II design - 18 *aa* and 18 *ab* (equal numbers of rams and ewes) from DD sire 1 and 3 *ac* ewes, 4 *ac* rams, 9 *aa* rams and 3 *aa* ewes from DD sire 2 were slaughtered commercially and meat samples taken and analysed as described by Morton *et al.*, (1997). Parameters measured were preslaughter liveweight, carcass weight, LD 24 h tenderness, *Psoas major* 24 h tenderness, LD calpastatin, μ- and m-calpain at 0 and 12 hours and 90 min and 24 hr LD pH. Data comparisons from each trial with p values less than or equal to 0.20 are shown.