the QTL for NEM1 is the same as that for FEC2 in the Woodlands Coopworth progeny test flock, nor is it known if the QTL alleles detected in the Romney, Perendale and Coopworth flocks are identical, variants or are derived from different loci altogether. Similarly, pleiotropic effects of these alleles on other traits have not been investigated. Definitive answers to these questions await linkage studies, but identification of the individuals to be mated and the mating design used will be guided by the results of these and similar analyses.

# A candidate gene approach to animal quality traits

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## ABSTRACT

A variety of animal quality traits, particularly those associated with meat and wool production and disease resistance, have been the subject of sophisticated genetic analyses. Investigation of the biochemistry and physiology underlying disease or production traits has implicated particular proteins and hence genes as "candidates" for having major phenotypic effects. This makes the candidate gene approach a useful complement to animal genome mapping of markers and QTLs. We have applied the candidate gene approach for the sheep quality traits of meat tenderness and footrot resistance.

A three allele system detected by polymerase chain reaction - single strand conformational polymorphism (PCR-SSCP) has been observed for the ovine calpastatin gene. Calpastatin is the specific inhibitor of the ubiquitous calcium-dependent proteases μ-calpain and m-calpain. There is very strong population genetic and protein assay data in cattle to inversely correlate post-mortem calpastatin levels with meat tenderness. Assay of post-mortem calpastatin levels in aging lamb confirms an important regulatory role for calpastatin in sheep meat aging. Data sets from preliminary experiments testing the association of the three allele ovine calpastatin system with meat tenderness values and other meat quality characteristics show significantly different fillet tenderness, early post-mortem calpastatin and μ-calpain levels between ewes with different calpastatin genotypes. The early post-mortem calpastatin levels and the *longissimus dorsi* pH at 24 hr post-mortem of sheep representing two different genotypes was also significantly different.

The major histocompatibility (MHC) proteins of vertebrates have a role in presenting peptide fragments from pathogenic organisms to the systemic immune system. It is hypothesised that a component of resistance in sheep to footrot is based on an effective systemic immune response and hence controlled by MHC presentation. Sixteen alleles have been identified at the ovine MHC class II *DQA2* locus and 8 at the *DQA1* locus. A significant (p=0.001) association between footrot status and *DQA2* genotype was observed in a halfsib family challenged with the disease under standardised field conditions.

**Keywords:** candidate gene; sheep; meat tenderness; calpastatin; footrot resistance; MHC proteins.

## INTRODUCTION

Understanding of the molecular genetics of production animals is developing rapidly and has enormous potential in helping to improve the quality and magnitude of animal production. Much of the activity in this field involves mapping quantitative trait loci (QTLs) using molecular approaches that scan entire animal genomes and then allow prediction of regions likely to contain QTLs for specific phenotypes. Such "whole genome" approaches are particularly useful when there are no obvious candidate genes for a specific trait.

There are phenotypes where, because of previous biochemical and physiological investigation or theoretical extrapolation from other species at least one candidate gene can confidently be predicted. In these cases a candidate gene approach to understanding the genetics of a particular animal quality trait can be justified, without prior genome scanning for QTLs. The candidate gene approach has potential advantages in terms of the time and effort that are required for "whole genome" approaches using large scale linkage analyses and gene mapping.

This study reports two examples of a candidate gene approach to characterising the inheritance of traits in sheep. The traits are meat tenderness and footrot resistance with the candidate genes under examination being the calpastatin gene (CAST) and the class II major histocompatibility (MHC) genes respectively.

The calpain-calpastatin system of calcium-dependent neutral proteases (μ- and m-calpains) and their specific

inhibitor, calpastatin, have emerged as having the primary role in the post-mortem myofibrillar degradation responsible for meat tenderisation (Koohmaraie *et al.*, 1995; Dransfield , 1994). In beef cattle the calpastatin gene has been implicated as an important gene influencing ultimate meat tenderness (Shackelford *et al.*, 1994). It is hypothesised that variation in calpastatin levels post-mortem ovine muscles is at least in part due to variation in the CAST locus. As yet, polymorphic variation in the gene (Koohmaraie *et al.*, 1995) has not been found to be associated with significant differences in meat tenderness (Lonergan *et al.*, 1995).

There is evidence of a genetic basis to variation in resistance to footrot. Heritability has been calculated as 0.28 for NZ Romney sheep under natural challenge (Skerman *et al.*, 1988) and 0.31 for Merino sheep under artificial challenge (Raadsma *et al.*, 1990). Two studies have revealed associations between the MHC complex of sheep (*MhcOvar*) and footrot resistance. Outteridge *et al.* (1989) showed that two class I antigens, appeared to be significant in influencing susceptibility or resistance, while Litchfield *et al.* (1993) utilised Southern hybridisation analysis and noted a number of potentially useful associations with the ovine MHC class II *DR* region.

We have investigated a half-sib family challenged with ovine footrot and detail the association of resistance with a specific haplotype at the *MhcOvar* class II region.

## CAST - a candidate gene for ovine meat tenderness

Polymorphic variation in the exon 1C/1D region of ovine CAST was assessed using the polymerase chain reaction-single stranded conformational polymorphism (PCR-SSCP) genotyping strategy reported by Roberts *et al.* (1996). To date three alleles (***a***, ***b*** and ***c***) have been detected using SSCP. The relative frequencies of CAST alleles in four different flocks are shown in Table 1. Allele ***a*** is predominant in all flocks with varying frequencies for ***b*** and ***c***. All possible combinations of the three alleles, with the exception of ***c*** homozygotes have been found.

Sequence analysis of animals (n = 3) homozygous for alleles a and b revealed the amplimers were 612 bp in length and six single base differences between the ***a*** and ***b*** allele sequences. All the differences occur within intron 12. Five of the six sequence differences lead to changes to restriction endonuclease (RE) recognition sites (Fig. 1). The sixth polymorphism lies within two overlapping regions of sequence symmetry, neither of which are recognition sites for a characterised RE. None of the sequence differences appear to alter sequence features that would affect the efficiency of intron splicing, but they may affect message editing, mRNA stability or another processing reaction.

**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.

**FIGURE 1:** Nucleotide sequence features of the ovine calpastatin gene exon 1C/1D region. A schematic diagram of the 612 bp amplimer showing the positions in nucleotides from the 5' end of the upstream PCR primer *calpsd* (Roberts *et al.*, 1996) of the nucleotide sequences differences between alleles ***a*** and ***b***. The nucleotide differences are shown above (allele ***a***) and below (allele ***b***), as are two restriction endonucleases which have sites in the amplimer of one allele only. These enzymes, *Msp*I and *Nco*I, have been used in a PCR-RFLP to differentiate ***aa***, ***ab*** and ***bb*** amplimers (data not shown).

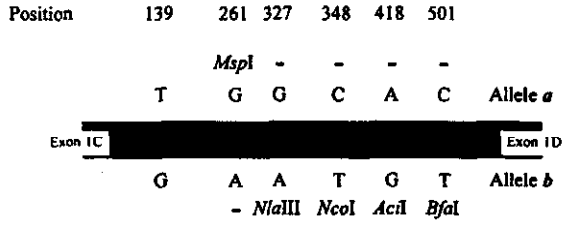


**TABLE 2:** Summary and interpretation of the meat quality characteristics data set from the slaughter trial.

| Meat Quality Characteristic | Putative Association with Genotype | Statistical Significance* | Comments |
|---|---|---|---|
| **Tenderness/pH** | | | |
| Psoas major shear force in ewes | aa<ab<ac | p<5.1% | Fillet from ac animals significantly tougher than that from other genotypes |
| LD shear force in ewes | aa<ab<ac | p<16.6% | Fillet from ac animals marginly tougher than that from other genotypes |
| LD pH (24 h) | aa<ab | p<1% | Highly significant |
| | | | Potentially commercially valuable |
| | | | Doesn't correlate with tenderness data as well as might be expected |
| **Enzymes** | | | |
| LD calpastatin activity at 0 time | aa<ac | p=4.3% | Possible explanation for both rams and ewes tougher meat |
| LD calpastatin activity at 0 time | aa<ab<ac | p=4.3% | Possible explanation for ac ewes tougher meat |
| LD μ-calpain at 1 h | aa>ab>ac | p=2.8% | Effect on calpastatin to μ-calpain ratio. |

*ANOVA analysis using MINITAB 9.2.

## CAST polymorphism and meat quality

Genotyped Dorset Down sheep were slaughtered 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 ***cc*** homozygotes meant that only comparisons between ***aa*** (n = 11), ***ab*** (n = 13) and ***ac*** (n = 5) genotypes were possible. Sheep carrying a *b* allele 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 ($p<0.05$). With virtually all the measured parameters, rams had to be excluded to achieve significance when plotting meat quality against genotype, suggesting an over-riding sex effect independent of CAST genotype. The exception was LD calpastatin activity at 0 time ($p = 0.04$) for all animals when genotype was the variable parameter. The differences in shear force were paralleled by changes in components of the calpain-calpastatin system.

## Footrot Challenge and MHC Typing

Animals from the Broomfield Corriedale Breeding Group, Waipara, were used, a flock in which resistance to footrot is the primary selection objective (Skerman and Moorhouse, 1987, Moorhouse and Skerman, 1988, Outteridge *et al.*, 1989). A single Corriedale sire that had not been exposed to footrot was imported from Australia and introduced into this previously closed flock. Over two breeding seasons, this sire was mated with groups of resistant Broomfield ewes. Over late-autumn/winter the ram hogget progeny were collectively challenged with footrot as outlined below.

Ten footrot-infected sheep were run with 36 ram hogget progeny from the sire to provide a disease challenge. All animals were feed-lot grazed as a single mob at a density of approximately 50 $ha^{-1}$ with *ad-lib* pellet feeding. After four weeks all animals were scored for footrot and moved to a second feed-lot where they were maintained for a further four weeks before foot lesions were again scored using the scoring system of Skerman *et al.* (1988). DNA, isolated from ear skin tissue of the progeny by phenol-chloroform extraction, was genotyped by Southern hybridisation with ovine-specific MHC class II probes as described by Escayg *et al.* (1996). Animals were typed at the *DQA1*, *DQA2*, and *DQB* loci defining a haplotype.

At the second inspection two main groups of animals were identified, those which showed no clinical signs of infection (n=8) and those which demonstrated severe footrot with at least one foot possessing a grade 4 lesion (n=25). Three animals demonstrated the ability to self-cure, showing signs of footrot at the first inspection but not at the second.

Each of the progeny was DNA-typed to determine which paternal haplotype it had inherited. This could not be done for one animal which had an identical genotype to the sire. Association between the haplotype inherited from the sire and the disease status of the progeny was investigated for the remaining 35 animals by chi-square analysis. Associations were investigated by comparing resistant, susceptible and self-curing groups, and also by comparing combined resistant and self-curing with susceptible animals. Self-curing animals were also excluded from analysis by restricting the comparison to resistant and susceptible animals.

A highly significant association ($p = 0.005$) between haplotype and footrot status was observed within the half-sib family when progeny were classified as resistant, susceptible and self-curing. The probability increased ($p = 0.002$) when the self-curing and resistant animals were combined and became even more significant ($p = 0.001$) when the self-curing animals were excluded from analysis.

This study has identified a potentially useful association between the ovine MHC class II region and resistance to footrot. It confirms previous studies which have provided some evidence for the involvement of the ovine MHC region in modulating the response of sheep to footrot (Outteridge *et al.*, 1989, Litchfield *et al.*, 1993). Work is proceeding on other half-sib families from different breeds.

## CONCLUSION

Both these examples of the candidate gene approach to characterising the inheritance of animal quality traits show that promising results have been obtained using this strategy with relatively modest resources. Good genetic records for the animals used in such studies is essential and the existence of selectively bred flocks has help provide the diversity which is needed to link a specific phenotype with a specific genotype. The candidate gene approach and "whole genome" approaches have complementary roles to play in the study of animal quality traits each having its place dependent on the nature of the trait under investigation.