SINGLE NUCLEOTIDE POLYMORPHISMS IN THE CALPAIN AND CALPASTATIN GENES AND BEEF TENDERNESS

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Introduction
Currently, the biochemical pathway with the most evidential support for involvement in post-mortem tenderisation is that of the calpain family of proteases. Calpain I and II act to break down the myofibrillar proteins via calcium-dependent proteolytic degradation post-mortem and thus increase meat tenderness. Calpastatin (CAST) is the inhibitor of calpain I and II activity, regulating post-mortem proteolysis. Increased CAST activity is associated with less tender meat (Koohmaraie *et al.*, 1995). If single nucleotide polymorphisms in these genes are found to display an association with beef tenderness, they could provide the basis of a diagnostic test for tenderness or improve breeding programmes through marker assisted selection. SNP variants exist in exons 9 and 14 of the calpain I gene that alter the protein sequence of calpain I (Page *et al.*, 2002). These have been found to be associated with a measure of beef tenderness, i.e. Warner-Bratzler shear force (WBSF) in resource populations (Page *et al.*, 2002, Casas *et al.*, 2006). A restriction fragment length polymorphism (RFLP) exists in Calpain II (Zhang *et al.*, 1996) that may also be associated with beef tenderness. These markers have been tested for association with WBSF in bovine *M. longissimus dorsi* in the Irish herd and a SNP in exon 9 was significantly associated with this parameter (Costello *et al.*, 2004). Following on from this work, our aim was to determine the frequencies of SNP variants at candidate loci in the calpain family for beef tenderness in different breeds in the Irish herd. A recently-identified SNP allele in the CAST gene has been shown to have a significant association with reduced WBSF values and a reduction in the proportion of unacceptably tough steaks (Schenkel *et al.*, 2006). The aim of this research was also to test this marker for association with WBSF, in Irish bovine *M. longissimus dorsi*.

Materials and Methods
DNA was isolated from 25mg of *M. longissimus dorsi* muscle (on which tenderness was also characterised) and thawed blood samples using the QIAamp® DNA minikit. Muscle samples were used in both the association analysis and the frequency study while the blood samples which were procured from Irish AI stations were used solely to determine genotype frequencies. Tenderness scores were measured by WBSF on steaks from the posterior end of the LD using a modified method of Shackelford *et al.* (1991). Polymerase Chain reaction (PCR) was carried out using primers specific for calpain I exon 9 and 14 and the partial calpastatin gene (Schenkel *et al.*, 2006). Restriction digestions were carried out using the restriction enzymes *Btg*I (calpain I, exon 9), *Dpn*II (calpain I, exon 14) and *Rsa*I (CAST). Digests were subjected to electrophoresis on 2% agarose gels, photographed under UV transillumination and genotypes scored. Association analysis was performed between the observed CAST genotypes and WBSF at day 14 post mortem using the GLM procedure of GenStat.

Results and Discussion
Genotype frequencies for all breeds tested are presented in Table 1. The GA genotype at the Calpain I, exon 9 locus, which is significantly associated with reduced WBSF (Costello *et al.*, 2004), was the minor genotype at that locus in the Irish herd.

Table 1: Breed specific frequencies (%) for Calpain I and Calpain II genotypes- genotypes were assigned following published designations.

Breed (n)	Calpain I, exon 9		Calpain I, exon 14		Calpain II		
	GG	GA	VV	VI	AA	AB	BB
Friesian (64)	95.3	4.7	80.8	19.2	16.7	64.8	18.5
Belgian Blue (11)	100.0	-	100	-	27.3	54.5	18.2
Limousin (11)	81.8	18.2	81.8	18.2	45.5	54.5	-
Charolais (9)	77.8	22.2	100	-	22.2	44.4	33.3
Aberdeen Angus (7)	100	-	83.3	16.7	-	85.7	14.3
Others (17)	82.3	17.7	93.3	6.7	31.3	50.0	0.063

The GA genotype at the Calpain I, exon 9 genotype varied in frequency among breeds and was absent from the Belgian Blue and Aberdeen Angus samples tested. This indicates the potential of marker-assisted selection, based on the A allele as a functional marker, to significantly increase beef tenderness in the Irish herd by selectively breeding animals with favourable genotypes in breeding programmes. There was variation among genotype frequencies for the VI genotype at the exon 14 locus which was absent from the Belgian Blue and the Charolais breeds, although this allele was not significantly associated with tenderness, while there was less variation in genotype frequency among breeds for the Calpain II genotypes.

A significant association was observed between WBSF and genotypes at the calpastatin locus in the Irish herd ($p=0.015$). The CC genotype had a LSM shear force of 41.8 and the GG genotype had a LSM shear force of 51.6: the GC genotype had an intermediate LSM shear force of 45.5. This association concurs with results of recent studies (Schenkel *et al.* 2006). The overall frequency of the CC genotype was 0.41 (Table 2), again indicating the potential for incorporation into marker assisted breeding programmes. However, samples with potentially confounding factors such as extreme values of sarcomere length and intramuscular fat (IMF) should be excluded in a controlled sample set for future analysis. Care must also be taken when selecting against the alternative alleles at these loci, as they may contribute positively to other quantitative traits not examined here.

Table 2: Observed genotypic frequencies (%) in Irish bovine *M. longissimus dorsi*.

Gene	Calpain I, exon 9 (n=284)			Calpain I, exon 14 (n=284)		Calpain II (n=284)			Calpastatin (n=196)		
	GG	GA	AA	VV	VI	AA	AB	BB	CC	CG	GG
Frequency	76.5	23.2	0.4	75.6	24.4	15.2	53.0	31.9	0.41	0.47	0.13

Conclusion

The calpastatin CC genotype was observed to have an effect on tenderness in the Irish herd. Both this genotype and the A allele at the calpain 1, exon 9 locus may have potential for incorporation into marker-assisted breeding programmes to improve the consistency of beef quality in the Irish herd. In particular, the calpain 1 allele is currently at low frequency in the Irish herd, thereby offering considerable potential as a functional marker to enhance beef tenderness in the Irish population.

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