ANALYSIS OF THE RELATIONSHIP BETWEEN DNA POLYMORPHISMS IN CANDIDATE GENES AND BEEF TENDERNESS

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Background

The factors underlying meat quality, while poorly understood, have a molecular basis, which are responsive to nutrition, environment and animal breeding. Major progress is being made worldwide in identifying variations in DNA (polymorphisms) which are related to genes influencing meat quality, growth and carcass yield. Some of these polymorphisms may have the potential to serve as markers of meat quality. These include polymorphisms in the calpain I and II and growth hormone genes. The calpain family are postulated to affect meat quality by initiating muscle protein degradation. Exons 9 and 14 of the calpain I gene were found to contain single nucleotide polymorphisms (SNPs), predicted to alter the protein sequence of calpain I. Analysis of these polymorphisms with Warner Bratzler shear force (WBSF) values showed that a relationship existed between the alleles containing the SNP and decreased meat tenderness (Page et al., 2002). A restriction fragment length polymorphism (RFLP) in the calpain II gene has also been identified (Zhang et al., 1996), which may have an effect on meat quality. Growth hormone has proven to be the major regulator of postnatal growth and metabolism in mammals and thus affects growth rate, body composition, health, milk production, and aging by modulating the expression of many genes. PCR-RFLP analysis was used to identify a polymorphism in intron 3 of the bovine growth hormone gene (Zhang *et al.,* 1993). A population of Piedmontese cattle was genotyped for this growth polymorphism and an association was found between WBSF at day 11 post-mortem and the polymorphic allele of bovine growth hormone (Di Stasio *et al.,* 2003).

Objective

The objective of this study was to evaluate the association of polymorphisms in bovine Calpain I exon 9 and 14, Calpain II and Growth Hormone genes with tenderness in Irish bovine *M.longissimus dorsi*.

Materials and methods

Genomic DNA was isolated from 25mg of *M. longissismus dorsi* muscles (n=284) on which quality attributes were also characterised, using the QIAamp® DNA minikit. Tenderness was measured by WBSF (Shackelford, 1991). Compositional analysis was conducted to determine any variation in intramuscular fat, protein and moisture levels. Sarcomere lengths were determined according to the laser diffraction method (Cross *et al.,* 1980). Polymerase Chain Reaction (PCR) was performed using primers specific for calpain I exon 9 and 14 (Costello et al., in preparation), calpain II (Zhang et al., 1996) and growth hormone genes (Zhang et al., 1993). Restriction digests were carried out using the restriction enzymes *Btg*I (calpain I exon 9), *DpnII* (caplain I exon 14) *HhaI* (calpain II) and *Msp*I (growth hormone). Digests were analysed on agarose gels using electrophoresis (Figure 1).

Associations between the four polymorphisms and WBSF day 14 values were tested using the GLM procedure of SAS. Association analysis was performed between the observed genotypes and WBSF at day 14 post-mortem. In a controlled data set, confounding factors were excluded, in particular animals with extreme values of sarcomere length and intramuscular fat (IMF). For sarcomere length, cut off points of 1.4µm and 1.85µm were employed. Samples with percentage of IMF greater than 5% were also removed from the sample set.

Results and discussion

A significant association was observed between the calpain I exon 9 genotypes and WBSF in both the controlled ($P = 0.0333$) and uncontrolled ($P = 0.0033$) data-sets (Tables 1 and 2). Animals with the

GA genotype exhibited decreased WBSF when compared to animals with the GG genotype. No association was observed between WBSF and calpain I exon 14 genotypes in either sample set. The V to I transition occurred in domain III of the protein, representing a conservative substitution of non-polar amino acids with no apparent function in terms of calpain enzyme activity. In contrast the G to A transition, which occurs in domain II of the protein, which has been identified as the proteolysis domain, thus this polymorphism could alter calpain I enzyme activity.

No significant association was observed between the growth hormone or calpain II polymorphisms, and WBSF. Di Stasio *et al.,* (2003) observed a significant association between the C allele of growth hormone and WBSF at day 11, but cautioned that the statistical model used to calculate the effect could lead to biased estimates of single gene effects, and that the highly unbalanced genotype distribution observed was undesirable for association analysis. However, our observed genotypic and allelic frequencies indicate that there is a higher frequency of C alleles over D alleles in general. Low frequencies for the D allele have previously been observed across Northern European breeds (Lagziel *et al.,* 2000), suggesting difficulties in finding a "balanced" genotype distribution in the Irish national herd.

Conclusions

It was found that the calpain 1 exon 9 genotypes had a statistically significant association with WBSF such that animals with the GA genotype exhibited decreased WBSF and increased tenderness, when compared to animals with the GG genotype. This observation concurs with that of earlier studies (Page *et al.,* 2002), strongly suggesting that this polymorphism is a functional marker for increased beef tenderness. None of the other genotypes examined were observed to have an effect on tenderness.

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Figure 1: Agarose gels showing RFLP patterns and corresponding genotypes for each of the polymorphisms. M = marker, UC = uncut fragment, bp = base pairs.

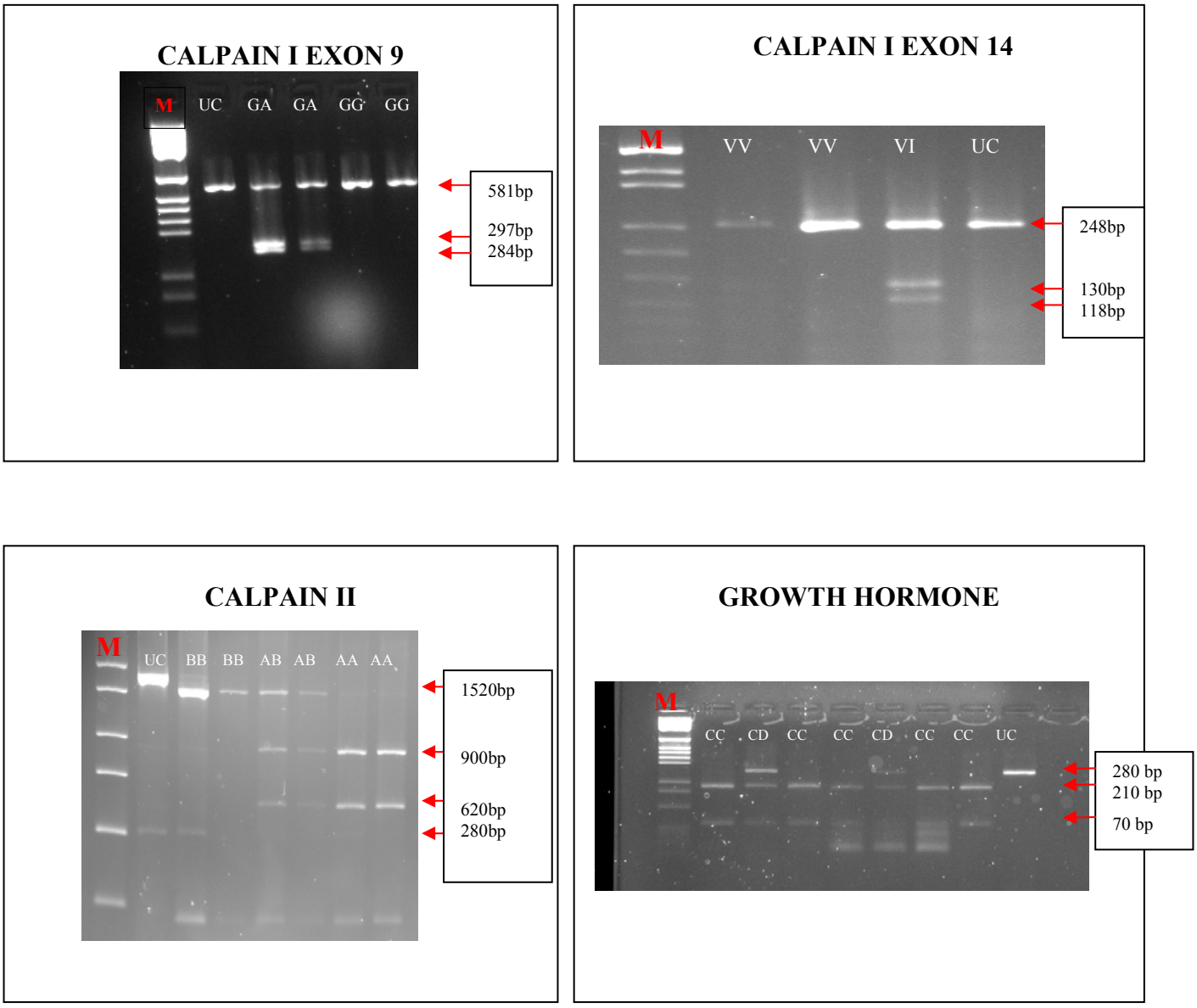

Table 1: Association analysis between observed genotypes and Warner Bratzler shear force (WBSF) values in bovine *M.longissimus dorsi* - Uncontrolled data-set

Gene	Genotype	Warner-Bratzler Shear Force (N) (mean±SDEV)	n	*P*
Calpain I, exon 9	**GG**	49.42 ± 23.61	208	0.0033
	GA	39.05 ± 11.50	63	
	AA	34.15	1	
Calpain I, exon 14	**VV**	46.20 ± 19.3	177	NS
	VI	51.82 ± 30.58	57	
Calpain II	**AA**	46.02 ± 16.36	41	NS
	AB	43.80 ± 16.63	143	
	BB	49.06 ± 27.58	86	
Growth Hormone	**DD**	33.53 ± 11.44	5	NS
	CD	46.61 ± 27.12	43	
	CC	47.59 ± 20.89	220	

NS = non significant; N = newtons

Table 2: Association analysis between observed genotypes and Warner Bratzler shear force (WBSF) values in bovine *M.longissimus dorsi* - Controlled data-set

Gene	Genotype	Warner-Bratzler Shear Force (N) (mean±SDEV)	n	*P*
Calpain I, exon 9	**GG**	51.34 ± 17.60	82	0.0333
	GA	42.24 ± 12.81	27	
	AA	34.15	1	
Calpain I, exon 14	**VV**	47.60 ± 15.04	77	NS
	VI	48.62 ± 15.74	28	
Calpain II	**AA**	46.68 ± 17.36	18	NS
	AB	46.90 ± 16.77	58	
	BB	50.72 ± 16.19	34	
Growth Hormone	**CC**	48.62 ± 16.24	99	NS
	CD	50.38 ± 21.72	13	

NS = non-significant. N = newtons

Note: Genotypes were assigned according to published literature. Genotypes for calpain I exon 9 and exon 14 refer to the coded amino acid (ie Exon 9: G refers to the amino acid glycine, A refers to alanine; exon 14: V refers to valine, I refers to isoleucine). Genotypes for calpain II and growth hormone were arbitrarily assigned.