

**ASSOCIATION OF POLYMORPHISMS IN CANDIDATE GENES WITH CARCASS AND TASTE PANEL ASSESSED MEAT QUALITY TRAITS IN A COMMERCIAL POPULATION OF ANGUS-SIRED BEEF CATTLE**

**J.L. Gill<sup>1</sup>, S.C. Bishop<sup>1</sup>, C. McCorquodale<sup>1</sup>, J.L. Williams<sup>2</sup> and P. Wiener<sup>1</sup>**

<sup>1</sup>The Roslin Institute and R(D)SVS, University of Edinburgh, Edinburgh, UK,  
<sup>2</sup>Parco Tecnologico Padano, Lodi, Italy

**SUMMARY**

Associations between polymorphisms in candidate genes and economically important meat quality traits were assessed in a commercial population of Aberdeen Angus-cross animals. A number of traits were measured including 20 carcass and sirloin measurements recorded shortly after slaughter and also following maturation, 1 mechanical measure of tenderness and 7 taste panel assessed sensory traits. Polymorphisms tested included those in the calpain, leptin and myostatin genes. A number of significant and potentially important associations were found. An association was observed between a SNP in the calpain gene and meat tenderness, measured by both the tenderometer and the taste panel ( $P=0.01$  for both), where the allele associated with tenderness was also associated with heavier hindquarters. Additionally we found significant associations between a leptin SNP and overall liking ( $P=0.02$ ) and a DGAT1 SNP and sirloin fat depth ( $P<0.05$ ).

**INTRODUCTION**

Meat quality is of great importance to the beef industry, but quality traits such as tenderness and juiciness often have low heritabilities and can only be measured post-slaughter, reducing the effectiveness of traditional phenotype-based breeding strategies. Marker-assisted selection has the potential to solve these problems and several markers associated with quality traits have recently been discovered in genes such as calpain (*CAPN1*), calpastatin (*CAST*), bovine growth hormone receptor (*BGHR*) and acylCoA:diacylglycerol acyltransferase 1 (*DGAT1*). Before such information can be used in breeding programmes, unbiased and independent validation studies should be carried out to establish whether the effects are found in the breed and population of interest. The aim of this study was to test for associations using polymorphisms in various candidate genes in a sample of over 400 Scottish Aberdeen Angus-cross animals collected through a commercial abattoir.

**MATERIALS AND METHODS**

**Data.** The sample set used to test the majority of polymorphisms consisted of 443 commercial crossbred beef cattle with purebred Aberdeen Angus sires sourced through the Scotbeef abattoir (Bridge of Allan, Scotland). Cattle originated from 14 breeder finisher farms (i.e. farms where animals are bred and finished on the same farm) and were selected to be representative of British commercial cattle slaughtered for beef production, i.e. a mix of heifers and bullocks (castrates). The data set used to test the myostatin polymorphism included a further 93 animals from the same source. Twenty carcass and sirloin traits, such as hot carcass weight, hindquarter weight, sirloin weight and eye muscle area were measured shortly after slaughter and also following maturation. In addition, 7 taste panel assessed sensory traits were measured, as well as a mechanical measure of tenderness, recorded using a tenderometer machine.

**Genotyping.** Samples were genotyped for 9 polymorphisms in 6 different genes. The genes (and polymorphisms) were *CAPN1* (*CAPN316*, *CAPN4751*), *CAST* (*UoGCAST*), Leptin (*UASMS1*, *UASMS2*, *Exon2FB*), *BGHR* (1 single nucleotide polymorphism (SNP)), *DGATI* (1 dinucleotide substitution) and myostatin (*GDF-8*) (an 11 base-pair deletion referred to as *del11*). All animals with phenotype information were genotyped, as were all available sires.

**Data Analysis.** The associations between the different genotypes of each SNP and the various traits recorded were evaluated using a single marker mixed model association analysis. Data were analyzed by fitting a linear mixed model using the restricted maximum likelihood method (REML) provided in Genstat, release 10 (Payne *et al.* 2007). The statistical model included fixed effects of farm, genotype, sex and the genotype-sex interaction, and random effects of sire, slaughter date (panel date for the taste panel traits), interaction of sire and slaughter date (panel date for the taste panel traits) and interactions of sire and slaughter date (panel date for the taste panel traits) with the genotype/sex groups. These latter interactions took into account the possibility of genotype/sex effects varying with sire or slaughter date (panel date for the taste panel traits) or both. Additionally, random effects were constrained to be non-negative, i.e. effects that were estimated to be negative were set to zero. Statistical significance for the fixed effects was determined using approximate F-statistics with denominator degrees of freedom estimated in the Genstat REML procedure (Kenward and Roger 1997).

Additive effects and dominance deviations were calculated from the predicted genotype means. The additive effect was estimated as half the difference between the mean of the 2 homozygotes, and dominance was estimated as the deviation of the heterozygote from the mean of the 2 homozygotes (Falconer and Mackay 1997).

## RESULTS AND DISCUSSION

Using single-marker, mixed-model association analysis 5 of the polymorphisms were found to be significantly associated with one or more of the traits tested. These include the *CAPN316* SNP in the *CAPN1* gene, the *UASMS2* SNP in the Leptin gene, the *DGATI* SNP, the *BGHR* SNP and the *del11* polymorphism in the *GDF-8* gene. Table 1 shows the genotype means and P-values for these significant associations.

Significant associations were found between *CAPN316* genotype and meat tenderness, measured using both the tenderometer machine and the taste panel, which is in agreement with previous findings (Page *et al.* 2005). Animals with the CC genotype at this SNP had more tender meat ( $P=0.01$ ) as expected from the earlier study, however, they also had heavier hindquarters. No associations were found between the *CAPN4751* SNP, and tenderness, or between the *CAST* SNP and tenderness as previously documented (Casas *et al.* 2006). No associations were found between the SNPs in the leptin gene and carcass quality traits as had been previously shown (Nkrumah *et al.* 2005; Schenkel *et al.* 2005). However, the leptin SNP, *UASMS2*, was found to be significantly associated with overall liking ( $P=0.02$ ) measured by the taste panel, where panellists gave animals with the TT genotype significantly higher overall liking scores than animals with CC or CT genotypes. This SNP has previously been associated with both backfat thickness and marbling score with TT animals having higher values for both traits (Nkrumah 2005). Additionally, the present study showed a significant, although non-additive, association between this SNP and sirloin fat depth where animals with the CC genotype had the lowest fat depth. The *DGATI* polymorphism has been shown to be significantly associated with milk fat yield and fat percentage, with AA animals having increased levels of both (Grisart *et al.* 2005). The present study found that this polymorphism was associated with sirloin weight after maturation ( $P=0.04$ ) and sirloin fat depth ( $P<0.05$ ). In both cases the A allele was associated with the higher value indicating that the increase in sirloin weight was probably due to the increase in the depth of fat

*Breeding program design including MAS*

surrounding the muscle. Regarding the myostatin gene, there were no *GDF-8* homozygous *del11* animals in the population studied and only 39 *wt/del11* animals. However, as with previous studies that have shown that a single copy of the *del11* allele has effects on carcass characteristics (Wiener *et al.* 2002; Casas *et al.* 2004), the data reported here show that animals inheriting the mutant *del11* allele were heavier at slaughter ( $P<0.05$ ), with higher Conformation Class scores ( $P<0.05$ ) and heavier hindquarters ( $P<0.001$ ); they had heavier sirloins, both before ( $P<0.001$ ) and after ( $P<0.01$ ) maturation and larger eye muscle areas ( $P<0.05$ ), suggesting a general increase in muscle mass for those animals with a single copy of the *del11* allele. Bonferroni corrections for multiple hypothesis testing reduced the number of apparently significant associations; however with a moderately stringent correction (6 genes as SNPs in the same genes were found to be in strong linkage disequilibrium) those associations with P-values less than  $\sim 0.01$  remained significant.

**Table 1. Genotype means, standard errors and P-values for polymorphisms with significant trait associations**

Trait	SNP	Genotype means $\pm$ se			Nominal P-value
	<i>CAPN316</i>	CC	CG	GG	
Tenderometer score (kPa)		22.25 $\pm$ 1.16	24.24 $\pm$ 0.70	25.18 $\pm$ 0.67	0.01
Hindquarter weight (kg)		75.67 $\pm$ 1.57	72.05 $\pm$ 0.84	71.84 $\pm$ 0.77	0.04
Tenderness		6.00 $\pm$ 0.16	5.79 $\pm$ 0.08	5.63 $\pm$ 0.07	0.01
	<i>UASMS2</i>	CC	CT	TT	
Overall liking		5.59 $\pm$ 0.08	5.55 $\pm$ 0.08	5.80 $\pm$ 0.10	0.02
	<i>DGATI</i>	AA	AG	GG	
Sirloin weight after maturation (kg)		8.31 $\pm$ 0.46	7.17 $\pm$ 0.12	7.14 $\pm$ 0.11	0.04
Sirloin fat depth, mm		11.11 $\pm$ 1.65	6.62 $\pm$ 0.4	6.53 $\pm$ 0.33	0.05
	<i>BGHR</i>	AA	AT	TT	
Odour		5.64 $\pm$ 0.19	5.24 $\pm$ 0.08	5.16 $\pm$ 0.06	0.02
	<i>GDF-8</i>	<i>wt/wt</i>	<i>wt/del11</i>	<i>del11/del11</i>	
Hot carcass weight (kg)		314.7 $\pm$ 3.2	332.1 $\pm$ 6.6	-	0.01
Sirloin weight before maturation (kg)		7.25 $\pm$ 0.1	7.88 $\pm$ 0.2	-	0.001
Sirloin weight after maturation (kg)		7.17 $\pm$ 0.1	7.70 $\pm$ 0.22	-	0.01
Confirmation class		7.19 $\pm$ 0.15	7.92 $\pm$ 0.32	-	0.02
Eye muscle area (mm <sup>2</sup> )		11077 $\pm$ 152.2	11890 $\pm$ 362.8	-	0.02
Hindquarter weight (kg)		72.2 $\pm$ 0.64	77.0 $\pm$ 1.44	-	0.001

There were significant sex-genotype interactions for 6 of the significant trait-polymorphism combinations tested. Analyses of the mean trait value for each genotype in each sex for those trait-SNP pairs indicated that, for the majority of associations, the effect was primarily in female animals. The exception was the effect of *CAPN316* on tenderometer values which was observed in the male animals. Differences between male and female genotype effects were seen in 5 traits: taste panel assessed tenderness, weight of hindquarter, odour, sirloin weight after maturation and sirloin fat depth. Differences could be partly due to the limited number of females (135) in the analysis when compared to the males (308) although allele frequencies for both sexes are similar.

Alternatively, trait expression could be strongly correlated with fatness. Means for each sex showed that females tended to have higher fat class scores than males (data not shown). Therefore, it is possible that the female animals are more likely to express genetic differences in traits correlated with fatness.

## CONCLUSIONS

Polymorphisms in 6 genes were tested for their associations with various economically important traits in a commercial population of Aberdeen Angus-cross animals. The results presented here confirm some of the previously-documented associations, for example, the association between *CAPN316* genotype and tenderness, the most important quality trait for consumers. Furthermore, novel associations were identified which, following validation in other populations, could be incorporated into breeding programmes to improve meat quality. Finally, whilst some previously reported associations were not replicated in the current study, it is important to note that validation is dependent on the specific nature of the population screened and that genetic background may influence the size of the effect of a polymorphism. Validation failure may be due to a lack of true associations between the trait and marker but could also be caused by differences in SNP frequencies, different SNP marker-causative mutation linkage phases, genotype-by-environment interactions or epistasis (Dekkers 2004), or the way the trait is measured. Nevertheless, for those associations confirmed here, the additional validation instils confidence in using these markers in selection programmes for improved meat quality.

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