Association of SNPs in candidate genes with meat quality in crossbred cattle

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Introduction

Meat quality describes properties and perceptions of meat. For industry, cooking yield is also economically relevant. Many challenges face the meat industry and there is a need to identify genetically superior animals for these traits (Mullen *et al*., 2006). Tenderness, intramuscular fat (IMF) level and waterholding capacity are beef quality traits with moderate heritability, but which are difficult to improve by conventional selection. We aimed to test single nucleotide polymorphisms (SNPs) in candidate genes involved in pathways of relevance to meat quality, for association with these traits in crossbred *Bos taurus* cattle.

Four published SNP in candidate genes were selected. Protein kinase AMP-activated gamma3-subunit (PRKAG3) gene regulates AMPK activity in skeletal muscle and strongly influences glycogen metabolism. A SNP in this gene is influential on cook loss in pork (Milan *et al*., 2000, Ciobanu *et al*,. 2001). PRKAG3 is also expressed in bovine skeletal muscle and 7 SNP have been detected in 4 cattle breeds by Yu *et al*. (2005). Growth Hormone Receptor (GHR) exerts its effects on growth and metabolism by interacting with growth hormone. The GHR^A allele of an A/G polymorphism at position 257 in exon 10 was associated with higher drip loss values in beef aged 3 days (Di Stasio *et al*., 2005). Stearoyl-coA Desaturase (SCD) is a protein complex that is key in the synthesis of monounsaturated fatty acids (MUFA). The SCD^A allele at a SNP at position 702 in the open reading frame of the cDNA, contributed to higher MUFA percentage and lower melting point in IMF (Taniguchi *et al*., 2003). Finally, CAPN1 is a calcium-dependent, non-lysosomal cysteine protease – a number of SNP in CAPN1 have been associated with tenderness in a number of studies (e.g. Costello *et al*., 2007). The TT genotype of a SNP in CAPN1 intron 14 has been shown to be associated with higher percentage lean share in valuable cuts, compared to the CC genotype (Jusczuk-Kubiak *et al*., 2004).

Materials and methods

M. longissimus thoracis et lumborum (LTL) and *M. semimembranosus* (SM) muscle were collected from Irish cross bred cattle (n=130). Bovine Hunter L*a*b* colour parameters were measured on day 2 post mortem, using the Mini-scan XE. Composition was analysed using the CEM SMART Trac rapid moisture/fat analyzer. Warner Bratzler shear force (WBSF) measurements were carried out on day 14, according to Wheeler *et al.* (1996). Samples were collected for sensory analysis and stored at -20^0C. Drip loss was measured using a bag method (Honikel, 1998).

DNA was isolated from muscle tissue using the DNeasy® Blood and Tissue Kit (Qiagen). SNP were genotyped using Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) analysis and agarose gel electrophoresis in accordance with author's protocols (Yu *et al*., 2005; Di Stasio *et al*., 2005; Taniguchi *et al*., 2005; Jusczuk-Kubiak *et al*., 2004). Genotype and allele frequencies were calculated and association analysis performed between the observed genotypes with relevant meat quality traits using the GLM procedure of SAS. A number of covariates, including sex, age, breed type (beef/ dairy), factory (plant 1, plant 2) and slaughter period (spring, summer or winter) were included in the model.

Results and discussion

PCR-RFLP genotypes for all SNPs were determined, by visual assessment. Genotype frequencies were estimated (Table 1). Levels of significance, least-squares means and errors from the models including candidate SNPs are reported in Table 1.

Trait	Gene	P-value	Estimated mean per genotype Genotype (frequency)		
	GHR		AA (0.38)	AG (0.37)	GG (0.25)
IMF (SM)		0.0064	0.79±0.23	1.67±0.21	0.85±0.31
IMF (LTL)		0.0609	2.22±0.22	2.75±0.2	2.04±0.3
L (SM)		0.001	25.86±0.84	29.17±0.76	24.94±1.13
Moisture (SM)		0.0014	75.34±0.18	74.5±0.16	74.69±0.25
Nitrogen% (SM)		0.0188	22.1±0.12	22.25±0.11	22.61±0.16
L (LTL)		0.0043	27.44±0.79	29.81±0.72	26.09±1.08
Moisture (LTL)		0.1146	74.11±0.19	73.6±0.17	73.82±0.26
Nitrogen% (LTL)		0.0015	22.56±0.1	22.44±0.09	23.01±0.14
	PRKAG3		AA (0.38)	AG (0.37)	GG (0.25)
Cook (LTL)		0.018	31.22±0.32	31.71±0.35	30.23±0.43
Cook (SM)		0.073	33.15±0.33	34.08±0.38	33±0.45
	SCD		AA (0.53)	AG (0.28)	GG (0.19)
IMF (LTL)		0.0497	2.77±0.2	2.08±0.24	2.33±0.3
IMF(SM)		0.1364	1.47±0.21	1.09±0.25	0.81±0.32
L (LTL)		0.0292	28.51±0.72	29.51±0.86	26.04±1.08
a (LTL)		0.0082	24.05±0.7	23.41±0.83	27.21±1.06
b (LTL)		0.0191	12.29±0.23	12.34±0.27	13.32±0.34
L (SM)		0.0074	28.12±0.76	27.82±0.9	24.17±1.16
a (SM)		0.0127	24.63±0.77	23.67±0.91	27.81±1.18
b (SM)		0.0969	12.17±0.23	11.99±0.27	12.88±0.35
	CAPN1		CC(0.48)	CT(0.24)	TT(0.28)
Juiciness (LTL)		0.0395	5.42±0.14	5.14±0.2	4.88±0.17
Firmness (LTL)		0.0124	4.96±0.09	5.13±0.13	5.38±0.11
WB D14 (LTL)		0.2771	45.05±2.7	46.06±3.93	51.55±3.43
Texture (LTL)		0.3477	2.99±0.1	3.14±0.14	3.2±0.12
Firmness (SM)		0.8111	5.71±0.07	5.64±0.11	5.67±0.09
WB D14 (SM)		0.2183	52.06±1.56	55.78±2.24	51.2±1.98
Texture (SM)		0.4670	3.69±0.08	3.56±0.12	3.55±0.11

The GHR SNP was not associated with drip loss, in contrast to observations by Di Stasio *et al*., 2005, in which the GHR^A allele was associated with higher values of drip. An association was observed, however, between the GHR SNP and IMF level ($p<0.0064$) and with compositional traits (moisture and nitrogen %), indicating this SNP may have multiple roles and complex interactions with growth hormone (Di Stasio *et al*., 2005).

Previous research has reported a significant association of SNP variants in the PRKAG3 gene with cook loss and pork quality (Milan *et al*., 2000; Ciobanu *et al*., 2001). Here, SNP in this gene were found to influence the same trait in bovine LTL muscle ($p<0.018$), while there was no significant association in the SM muscle, though a tendency was observed ($p<0.0731$). The effect size of the SNP on cook loss in the LTL was relatively small (~1% mean difference among genotypes) but even such a difference could have economic significance for processors.

SNP variants in the SCD gene have been found to correlate with IMF levels and fatty acid profile (Taniguchi *et al*., 2005). In the present study, a SNP in the SCD gene was associated with IMF level in LTL muscle ($p<0.0497$), but not in SM muscle ($p<0.1364$). Also, several colour parameters were associated with the SCD SNP in both muscles, which suggests a potential link between percentage mono-unsaturated fatty acids in IMF and colour. This may be because MUFA influence susceptibility to oxidation.

The CAPN1 SNP was not associated with WBSF or sensory quality in LTL or SM muscle samples, although other SNP in the CAPN1 gene have been associated with tenderness in this population (Costello *et al*., 2007). CC individuals had a significantly higher mean score for juiciness ($p<0.0395$) and lower mean score ($p<0.0124$) for firmness in LTL muscle, when compared with TT, though this was not observed for the SM muscle.

Conclusions

These results indicate the potential of SNP variants in candidate genes to influence diverse palatability traits in crossbred cattle populations and that, in some cases, the effects can be muscle specific. Our data highlight the potential to define and optimize management systems through the incorporation of genomic technologies.

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