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Association of polymorphisms in candidate genes with colour, water-holding capacity, and composition traits in bovine *M. longissimus* and *M. semimembranosus*

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ABSTRACT

The objective of this study was to determine the association of single nucleotide polymorphisms (SNP) in selected candidate genes with sensory and technological meat quality traits in commercial cattle. SNP in seven candidate genes were genotyped in 130 crossbred *Bos taurus* cattle using PCR-RFLP. Reported associations between calpastatin (CAST) and Warner–Bratzler shear force and carboxypeptidase E (CPE) and intra-muscular fat were not confirmed. However, SNP in CAST, amp-activated protein kinase, gamma-3 subunit (PRKAG3), growth hormone receptor (GHR) and stearoyl coA desaturase (SCD) genes were significantly associated with colour traits ($p<0.05$). The PRKAG3 SNP was additionally associated with cook loss in *M. longissimus thoracis et lumborum* ($p<0.05$) and tended towards association in *M. semimembranosus* ($p<0.1$). An association with pH was identified for the SCD SNP ($p<0.001$). The GHR polymorphism was influential on moisture and intra-muscular fat in *M. semimembranosus* and protein content in both muscles ($p<0.05$). Only CPE was associated with sensory traits (flavour in *M. longissimus*, $p<0.01$).

1. Introduction

Consumer assessment of meat quality is defined by the characteristics of sensory experience: tenderness colour, juiciness, flavour, texture, as assessed by pH, intra-muscular fat content, colour, water-holding capacity, shear force and sensory analysis. Water-holding capacity has additional importance due to its ability to influence certain processed product attributes, such as consistency, colour, saltiness as well as its influences on lean yield; drip losses due to cutting can be 2–10%, which represents a significant economic loss to processors and retailers (Offer & Knight, 1988), and along with colour, it forms an important element of the consumer's perception of a meat cut (Sawyer, Apple, Johnson, Baublits, & Yancey, 2009).

The majority of meat quality traits have a genetic as well as environmental component (Dikeman et al., 2005) with heritability varying depending on the trait analysed (Wheeler, Cundiff, Shackelford, & Koohmaraie, 2004). A number of candidate genes have been identified as potentially relevant to beef sensory and technological traits. For example, the specific inhibitor of the calpain family of endogenous proteases, calpastatin (CAST), inhibits the normal tenderization of meat as it ages *post mortem* (Schenkel et al., 2006), maps to a QTL for shear force on BTA7 (Barendse et al., 2007) and is relevant to several water-holding capacity traits in pigs (Ciobanu et al., 2004) and juiciness in beef (Casas et al., 2006). The gene encoding the gamma-3 regulatory subunit of the amp-activated protein kinase gene (PRKAG3) maps to chromosome BTA2 (McKay, White, Kata, Loan, & Womack, 2003) and several QTLs for marbling are located on this chromosome. Polymorphisms in PRKAG3 have been found to be associated with glycogen content and allied meat quality traits across pork breeds (Ciobanu et al., 2001; Milan et al., 2000). SNP variants in the growth hormone receptor (GHR) gene have been linked to drip loss (Di Stasio, Destefanis, Brugiapaglia, Albera, & Rolando, 2005) and marbling score in beef (Han et al., 2009). Stearoyl coA desaturase (SCD) catalyses the desaturation of saturated fatty acids to monounsaturated fatty acids. Both gene expression and allelic variation in the gene have been shown to correlate with percentage of monounsaturated fatty acid in Japanese Black cattle (Taniguchi et al., 2004). The carboxypeptidase E (CPE) gene maps to chromosome 17 (Haegeman, Williams, Law, Van Zeveren, & Peelman, 2003) and is located near to a QTL for IMF (Barendse, Bunch, & Harrison, 2009). CCAAT/enhancer binding protein alpha (C/EBP-α) is a transcription factor that also plays an important role in lipid deposition as well as adipocyte differentiation (Shin, Kang, & Chung, 2007). Finally, heat shock protein 70 (HSP 70) acts as a molecular chaperone and protects the cell against exposure to lethal heat shock, which is capable of denaturing proteins (Grosz, Skow, & Stone, 1994), hence the gene has considerable potential relevance to tenderness and water-holding capacity.

Defining the link between the genome and quality attributes is a key step towards enabling the prediction and management of the ultimate quality of beef. The objective of the present study was to determine the association of sequence variation in selected candidate genes with technological and eating parameters of meat quality

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Table 1
The candidate polymorphisms selected, the original citation and RFLP method, as well as the minor allele frequencies observed in this sample.

Gene	Reference	PCR amplicon (bp)	PCR conditions[a]	Enzyme for RFLP	SNP (allele 1/2)	Minor allele (frequency)
CAST	Schenkel et al. (2006)	520	95 °C 10′ 8 cycles (94 °C 30 s, 63 °C 30 s; −1 °C per cycle, 72 °C 30 s), 27 cycles (94 °C 30 s, 55 °C 30 s, 72 °C 30 s)	*Rsa*I	C/G	G (0.25)
PRKAG3	Yu et al. (2005)	714	94 °C 5′ (94 °C 30 s, 57.5 °C 45 s, 72 °C 50 s) 30 cycles	*Ecor*I	A/G	G (0.35)
GHR	Di Stasio et al. (2005)	342	95 °C 5′,35 cycles (94 °C 45 s, 52 °C 50 s, 72 °C 50 s)	*Alu*I	A/G	G (0.42)
SCD	Taniguchi et al. (2004)	567	94 °C 2′, 35 cycles (94 °C 30 s, 51 °C 30 s, 72 °C 1′)	*Nco*I	A/G	G (0.39)
C/EBP-α	Shin et al. (2007)	421	94 °C 4′, 35 cycles (94 °C 30 s, 53 °C 30 s, 72 °C 45 s)	*Sma*I	A/C	A (0.43)
CPE	Haegeman et al. (2003)	~1500	94 °C 5′, 35 cycles (94 °C 30 s, 51 °C 30 s, 72 °C 45 s)	*Dde*I	A/B	B (0.21)
HSP 70-1	Grosz et al. (1994)	253	94 °C 5′, 40 cycles (94 °C 50 s, 51 °C 30 s, 72 °C 1′)	*Alu*I	A/G	Monomorphic

[a] For each PCR there was a final annealing step of 72 °C for 10 min.

determined in two muscles of commercial crossbred cattle. For some polymorphisms, associations have previously been shown with meat quality traits and we aimed to assess if published associations found extend to the crossbred European *Bos taurus* population using a resource of comprehensively phenotyped samples. Other polymorphisms have not yet been investigated for association with certain beef quality traits, yet are members of biological pathways hypothesised to be influential on those traits.

2. Materials and methods

2.1. Sample collection and beef quality measurements

M. longissimus thoracis et lumborum (LTL) and *M. semimembranosus* (SM) muscle were collected at slaughter from Irish cross bred cattle ($n=130$). Meat quality and sensory analysis is as described previously (Maher, Mullen, Moloney, Buckley, & Kerry, 2004; Pannier et al., 2009). Briefly, Hunter L^*, a^*, b^* colour parameters were measured on day 2 post mortem after 3 h blooming of the cut surface, using the Mini-scan XE (Hunter Associates Laboratory, Inc., Reston, VA, USA). Composition was analysed using the SMART system 5 (CEM, Matthews, NC, USA) for moisture and fat and the FP 328 (Leco, St. Joseph, MI, USA) for protein. Warner–Bratzler shear force (WBSF) measurements were carried out on day 14, according to Wheeler, Shackleford, and Koohmaraie (1996).

2.2. Genotyping

DNA was isolated from muscle tissue using the DNeasy® Blood and Tissue Kit (Qiagen, Crawley, UK) as per manufacturer's instructions. All PCR reactions were carried out in a final volume of 25 μl. The reactions consisted of: 12.5 μl distilled water; 5 μl of 5× magnesium free buffer (10 mM TrisHCl; 50 mM KCl; 0.1% Triton® X-100, Promega, Madison, WI, USA); 3 μl $MgCl_2$ (25 mM Promega, Madison, WI, USA); 0.75 μl of dNTP mix (10 mM stock mix); 1 μl of DMSO (Sigma Chemicals, St. Louis, MO, USA); 0.75 μl of each primer (100 pmol, Eurofins MWG Operon, Ebersberg, Germany); 0.25 μl Go*Taq*®Flexi DNA polymerase (5 U/μl, Promega, Madison, WI, USA) and 1 μl (approximately 50–150 ng) template DNA. Amplification conditions followed authors' protocols with some modifications outlined in Table 1 (Adamowicz, Pers, & Lechniak, 2005; Di Stasio et al., 2005; Haegeman et al., 2003; Schenkel et al., 2006; Shin & Chung, 2007; Taniguchi et al., 2004; Yu et al., 2005), and details are summarised in Table 1. Following PCR amplification, SNP were genotyped using Restriction Fragment Length Polymorphism (RFLP) analysis followed by visualisation on agarose gels. Genotypes were assigned by multiple operators in accordance with authors designations of observed patterns of RFLP bands.

2.3. Statistical analysis

Genotype and allele frequencies for polymorphic SNP were calculated in Genepop (Raymond & Rousset, 1995). Association analysis was carried out on the meat quality traits presented in Table 2 using the General Linear Model (GLM) procedure in SAS Version 9.1 (SAS Inst., Inc., Cary, NC) on 130 samples. 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. For those genes for which positive associations were identified, mean trait values for each of the genotypes were contrasted to test for significant differences using the Tukey–Kramer procedure in SAS.

3. Results and discussion

Mean and standard deviations for the meat quality traits in the commercial population studied are indicated in Table 2. Minor allele frequencies for polymorphic SNP ranged from 0.21 to 0.43 and are presented in Table 1. The SNP in the HSP70 gene was not segregating in the studied population ($n=40$ genotyped) and will not be discussed further. An association analysis was carried out for the remaining 6 genes. The GLM analysis revealed that all polymorphic markers tested, with the exception of C/EBPα, showed associations with aspects of meat quality, comprising 27 significant associations ($p<0.05$) and 8 suggestive associations ($p<0.1$), in total (Table 3). Significant associations were observed at the nominal level (0.05) between candidate SNP genotypes and meat quality traits including IMF %, colour, cook loss, pH and sensory quality. Following Bonferroni correction for multiple testing (Hochberg, 1988), 4 associations were deemed significant ($p<0.00026$) though this correction may be somewhat overly-conservative. These are highlighted in bold in Table 4. Not all published associations were confirmed (e.g. CAST and shear force) and in some cases, significant associations with meat

Table 2
Mean and standard deviations of meat quality parameters in the crossbred population studied.

Trait type	Trait	LTL mean ± standard deviation ($n=130$)	SM mean ± standard deviation ($n=130$)
Water-holding capacity	pH 48 h	5.54 ± 0.19	5.54 ± 0.14
	Cook loss (%)	31.03 ± 2.13	33.55 ± 2.24
Colour	Hunter *L* (day 2)	28.48 ± 5.00	27.57 ± 5.36
	Hunter *a* (day 2)	23.91 ± 5.32	24.90 ± 5.77
	Hunter *b* (day 2)	11.80 ± 2.10	11.88 ± 1.87
Technological	Warner–Bratzler shear force (Newtons)	49.02 ± 18.19	54.36 ± 9.80
	IMF %	2.32 ± 1.28	1.30 ± 1.34
	Protein (N) %	22.56 ± 0.62	22.20 ± 0.72
	Moisture %	73.96 ± 1.09	74.82 ± 1.07
Sensory (scores 1–8)	Tenderness	5.24 ± 0.92	4.82 ± 0.75
	Juiciness	5.05 ± 0.80	4.75 ± 0.79
	Flavour	3.79 ± 0.36	3.76 ± 0.32
	Firmness	5.20 ± 0.59	5.71 ± 0.49
	Texture	3.24 ± 0.65	3.62 ± 0.50
	Chewiness	3.51 ± 0.42	3.38 ± 0.38
	Overall acceptability	3.55 ± 0.52	3.43 ± 0.42