Meat Science 86 (2010) 270–275

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

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

W. Reardon [a], A.M. Mullen [a], T. Sweeney [b], R.M. Hamill [a,*]

[a] Teagasc, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland
[b] School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

ARTICLE INFO

Article history:
Received 23 November 2009
Received in revised form 11 March 2010
Accepted 11 April 2010

Keywords:
SNP
Water-holding capacity
Intra-muscular fat
Stearoyl coA desaturase
Beef

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

* Corresponding author. Tel.: +353 1 8059575; fax: +353 1 8059550.
E-mail address: ruth.hamill@teagasc.ie (R.M. Hamill).

0309-1740/$ – see front matter
doi:10.1016/j.meatsci.2010.04.013

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

Table 3
Summary of associations observed (associations and suggestive associations are underlined).

Trait type	Trait	CAST		PRKAG3		GHR		SCD		CEBP-alpha		CPE	
		LTL	SM	LTL	SM	LTL	SM	LTL	SM	LTL	SM	LTL	SM
Water-holding capacity	pH 48 h	0.0446	0.615	0.884	0.623	0.299	0.759	0.0001	0.0001	0.149	0.937	0.932	0.865
	Cook loss (%)	0.276	0.1224	0.018	0.073	0.435	0.355	0.387	0.233	0.537	0.27	0.361	0.0365
Colour	Hunter L (3 h)	0.0002	0.0017	0.0932	0.242	0.0043	0.001	0.029	0.0074	0.584	0.161	0.211	0.421
	Hunter a (3 h)	0.0002	0.0064	0.0365	0.268	0.135	0.145	0.0082	0.0127	0.388	0.179	0.117	0.139
	Hunter b (3 h)	0.02	0.0123	0.0385	0.037	0.224	0.826	0.019	0.0969	0.233	0.138	0.115	0.163
Technological	Warner–Bratzler shear force (Newtons)	0.42	0.874	0.9517	0.506	0.77	0.961	0.331	0.357	0.1001	0.0817	0.228	0.808
	IMF (%)	0.71	0.27	0.4621	0.877	0.0609	0.0064	0.0497	0.136	0.444	0.918	0.487	0.538
	Protein (N) (%)	0.38	0.767	0.585	0.499	0.0015	0.0188	0.705	0.167	0.235	0.352	0.978	0.599
	Moisture (%)	0.679	0.233	0.765	0.907	0.115	0.0014	0.085	0.552	0.0932	0.302	0.578	0.436
Sensory (scores 1–8)	Tenderness	0.92	0.496	0.1025	0.581	0.0619	0.526	0.856	0.825	0.815	0.254	0.57	0.865
	Juiciness	0.209	0.528	0.237	0.65	0.4029	0.682	0.742	0.343	0.651	0.652	0.841	0.192
	Flavour	0.295	0.44	0.812	0.374	0.39	0.431	0.398	0.608	0.757	0.792	0.0046	0.0612
	Firmness	0.92	0.673	0.127	0.255	0.162	0.235	0.638	0.425	0.796	0.775	0.18	0.486
	Texture	0.897	0.905	0.678	0.751	0.217	0.894	0.491	0.817	0.461	0.934	0.661	0.556
	Chewiness	0.429	0.275	0.916	0.353	0.597	0.681	0.329	0.811	0.267	0.737	0.583	0.937
	Overall acceptability	0.86	0.518	0.652	0.251	0.341	0.723	0.211	0.574	0.178	0.399	0.745	0.95

quality traits were identified that have not previously been described (e.g. SCD and colour). Tukey–Kramer analysis applied to nominally significant associations revealed significant genotype contrasts in many cases (Table 4). Data will be discussed for individual genes below.

3.1. Calpastatin gene (CAST)

There is considerable evidence that in different species, calpastatin activity in the post mortem muscle is highly related to meat tenderness via the inhibition of the endogenous cysteine peptidases, the calpains (Koohmaraie, Whipple, Kretchmar, Crouse & Mersmann, 1991; Sentandreu, Coulis & Ouali, 2002). Several reports have indicated that polymorphisms in the CAST gene are associated with shear force in *longissimus* muscle (Barendse, 2009; Casas et al. 2006; Schenkel et al. 2006). In the present study, genotypes of a SNP at position 257 bp in the CAST gene were not associated with tenderness, as assessed by WBSF or sensory panel (Table 3). This contrasts with findings of Schenkel et al. (2006) in which animals with the CC genotype produced more tender beef when compared with those characterised by GG or CG genotypes. It is often observed that SNP do not show consistent association with a trait when tested in unrelated populations, however this SNP has been correlated with shear force in several populations e.g. Van Eenennaam et al. (2007). The lack of observed association between CAST genotype and tenderness in the current study may suggest a different LD relationship between marker and putative causative mutation in these crossbred animals. A significant association was identified with variation at the calpain locus in the population of animals under study here (Costello et al., 2007) in which the homozygous glycine genotype was associated with higher shear force compared with the heterozygous glycine/alanine genotype, indicating the relevance of the calpain system to tenderness in this population.

The CAST SNP was, however, found to be significantly associated with pH in the LTL and highly significantly associated with colour parameters in both LTL and SM muscles. Animals with GG genotype displayed a significantly higher ultimate loin pH than those with CC or CG genotypes (5.68 in comparison to 5.57 and 5.59, $p<0.05$), whereas heterozygous individuals were lighter and redder in both loin and ham muscles ($p<0.05$). Although these ultimate pH values would be within the acceptable range, GG genotype animals may be more likely to produce dark-cutting, firm and dry beef compared with the other genotypes, depending on how the animals are managed. There are few similar studies in beef. Casas et al. (2006) identified an association between a different SNP in the 3′ untranslated region of CAST and juiciness in a US *B. taurus* population. In pork, there is positional genetic evidence supporting an influence on these traits (Malek et al., 2001) and CAST haplotypes have also been found to be positively correlated with several water-holding capacity traits, including cook loss and juiciness and displayed a tendency towards association with pH (Ciobanu et al., 2004). In pork at least, colour (in particular lightness) is well correlated with water-holding capacity (Joo, Kauffman, Kim, & Kim, 1995). The biological mechanisms underpinning the connection between calpastatin and colour and water-holding capacity traits could involve a link through Ca^{++} ion concentration and muscle contraction rate (Ciobanu et al., 2004). Additionally, if calpastatin has an influence on rate and extent of glycolysis and pH decline, this could influence colour and water-holding capacity. Melody et al. (2004) found correlations between water-holding capacity and calpain activity, calpain autolysis and protein degradation. They cite the hypothesis of Kristensen and Purslow (2001) that water-holding capacity in the early post slaughter period could be influenced positively by retained water in the muscle cell if there is rapid degradation of intermediate filaments such as desmin at this time. Our study confirms the importance of this gene in relation to aspects of water-holding capacity and colour.

3.2. AMP-activated protein kinase, gamma 3 subunit gene (PRKAG3)

The PRKAG3 gene codes for a regulatory protein influential on glycogen content in muscle, and SNP in the porcine gene have been shown to affect pork quality traits including ultimate pH, meat colour, water-holding capacity, drip loss, tenderness and cooking loss (Ciobanu et al., 2001; Milan et al., 2000). PRKAG3 is also expressed in bovine skeletal muscle and expression levels have been shown to correlate significantly with sensory flavour and juiciness (Bernard et al., 2007). Several SNP in the gene have been recently reported (Roux et al., 2006; Yu et al., 2005) although to our knowledge, only one study to date has examined polymorphisms in PRKAG3 in relation to beef quality (Ciani et al., 2007). Here, GLM analysis (Table 3) indicated that an A/G SNP reported by Yu et al. (2005) at position 3078 in intron 6 of the bovine gene was associated with cook loss in bovine LTL muscle ($p=0.018$) and also displayed a tendency towards association in the SM muscle ($p=0.073$). The rarer GG genotype exhibited a 1% lower cooking loss compared with both AA homozygotes and heterozygotes (Table 4), indicating a similar relationship between gene and trait in beef as in pork. This SNP was also associated with colour parameters (Hunter a and b in both LTL and SM). Preliminary analysis by Ciani et al. (2007) supports this finding, reporting a non-significant trend towards association of haplotypes in this gene with colour and water-holding capacity traits in beef. As proposed for pork, the relationship with these

Table 4
Significant associations ($p<0.05$) and tendencies ($p<0.1$) between genotypes and meat quality traits and least squares means per genotype.

Trait type	Trait	Muscle	Gene	*p*-value	Estimated mean per genotype[#]		
					11	12	22
Water-holding capacity	pH 48 h	LTL	CAST	0.0446	5.57 ± 0.036^{a}	5.59 ± 0.031^{a}	5.68 ± 0.044^{b}
		LTL	SCD	**0.0001**	5.63 ± 0.02^{a}	5.45 ± 0.03^{b}	5.59 ± 0.04^{a}
		SM	SCD	**0.0001**	5.57 ± 0.01^{a}	5.45 ± 0.01^{b}	5.58 ± 0.02^{a}
	Cook loss (%)	LTL	PRKAG3	0.018	31.22 ± 0.32^{a}	31.71 ± 0.35^{a}	30.23 ± 0.43^{a}
		SM	PRKAG3	<0.1			
		SM	CPE	0.0365	$33.48 \pm 0.28^{a,b}$	34.42 ± 0.59^{a}	32.47 ± 0.49^{b}
Colour	Hunter L (3 h)	LTL	CAST	**0.0002**	25.74 ± 0.962^{a}	29.33 ± 0.83^{b}	25.67 ± 1.14^{a}
		SM	CAST	0.0017	24.19 ± 1.00^{a}	27.57 ± 0.87^{b}	24.73 ± 1.19^{a}
		LTL	GHR	0.0043	27.44 ± 0.79^{a}	29.81 ± 0.72^{b}	26.09 ± 1.08^{a}
		SM	GHR	0.001	25.86 ± 0.84^{a}	29.17 ± 0.76^{b}	24.94 ± 1.13^{a}
		LTL	SCD	0.0292	28.51 ± 0.72^{a}	29.51 ± 0.86^{a}	26.04 ± 1.08^{b}
		SM	SCD	0.0074	28.12 ± 0.76^{a}	27.82 ± 0.9^{a}	24.17 ± 1.16^{b}
	Hunter a (3 h)	LTL	CAST	**0.0002**	27.06 ± 0.325^{a}	23.39 ± 0.28^{b}	26.64 ± 0.38^{a}
		SM	CAST	0.0064	27.25 ± 1.05^{a}	24.11 ± 0.91^{b}	26.76 ± 1.25^{a}
		LTL	PRKAG3	0.0365	25.41 ± 0.73^{a}	22.86 ± 0.82^{b}	$24.5 \pm 0.99^{a,b}$
		LTL	SCD	0.0082	24.05 ± 0.7^{a}	23.41 ± 0.83^{a}	27.21 ± 1.06^{b}
		SM	SCD	0.0127	24.63 ± 0.77^{a}	23.67 ± 0.91^{a}	27.81 ± 1.18^{b}
	Hunter b (3 h)	LTL	CAST	0.02	5.42 ± 0.18^{a}	5.35 ± 0.16^{b}	$5.35 \pm 0.21^{a,b}$
		SM	CAST	0.0123	12.93 ± 0.33^{a}	11.96 ± 0.29^{b}	$12.43 \pm 0.39^{a,b}$
		LTL	PRKAG3	0.0385	12.83 ± 0.23^{a}	12.01 ± 0.26^{b}	$12.47 \pm 0.32^{a,b}$
		SM	PRKAG3	0.0366	12.62 ± 0.23^{a}	11.81 ± 0.27^{b}	$11.99 \pm 0.32^{a,b}$
		LTL	SCD	0.0191	12.29 ± 0.23^{a}	12.34 ± 0.27^{a}	13.32 ± 0.34^{b}
Technological	Warner–Bratzler shear force (Newtons)	LTL	CEBP-alpha	<0.1			
		SM	CEBP-alpha	<0.1			
	IMF %	LTL	GHR	<0.1			
		SM	GHR	0.0064	0.79 ± 0.23^{a}	1.67 ± 0.21^{b}	0.85 ± 0.31^{a}
		LTL	SCD	0.0497	2.77 ± 0.20^{a}	2.08 ± 0.24^{b}	$2.33 \pm 0.3^{a,b}$
	Protein (N) %	LTL	GHR	0.0015	22.56 ± 0.11^{a}	22.45 ± 0.096^{a}	23.01 ± 0.14^{b}
		SM	GHR	0.0188	22.1 ± 0.12^{a}	$22.25 \pm 0.11^{a,b}$	22.62 ± 0.17^{b}
	Moisture %	SM	GHR	0.0014	75.34 ± 0.18^{a}	74.5 ± 0.17^{b}	74.70 ± 0.25^{b}
		LTL	SCD	<0.1			
		LTL	CEBP-alpha	<0.1			
Sensory (scores 1–8)	Tenderness	LTL	GHR	<0.1			
	Flavour	LTL	CPE	0.0046	3.82 ± 0.05^{a}	3.86 ± 0.06^{a}	3.73 ± 0.07^{a}
		SM	CPE	<0.1			

[#] Genotypes are as designated in Table 1, *p*-values in bold were significant after Bonferroni correction. Genotype means with different superscripts are significantly different at the 0.05 level.

traits in beef is likely to be related to the involvement of this gene as a regulator of muscle glycogen content.

3.3. Growth hormone receptor gene (GHR)

Growth hormone influences growth and metabolism by interacting with growth hormone receptor (GHR). The A allele of an A/G polymorphism at position 257 in exon 10 of GHR has previously been shown to be associated with higher drip loss values in Piedmontese beef aged 3 days (Di Stasio, Brugiapaglia, Destefanis, Albera, & Sartore, 2003). Here, this SNP was not associated with a related trait, cook loss, however, there was an association with moisture in SM ($p<0.01$) which was not previously reported by Han et al. (2009) or Di Stasio et al. (2003). GLM analysis also revealed significant associations with IMF level ($p=0.0064$) and colour ($p<0.001$, L, SM; $p=0.0043$, L, LTL). There is extensive evidence for the relevance of the growth hormone signalling pathway to fatness-related traits. For example, GHR has been shown to be associated with carcass fat in *B. taurus* populations (Tatsuda et al., 2008). GHR lies in a QTL region for milk fat content on chromosome 20 (Hoj, Fredholm, Larsen, & Nielsen, 1993; Viitala et al., 2006) and SNP in the gene associate with this QTL. A recent study reported a correlation between a different polymorphism in the GHR gene and marbling score, which is a trait that is closely correlated to IMF content (Han et al., 2009). Here, comparison of genotypes (Table 4) reveals that the heterozygous genotype is associated with significantly higher IMF % compared to the AA and GG genotypes. Muscle of individuals with GG genotype may maintain higher protein content, because a small but significant increase in nitrogen content in both muscles was observed in GG animals compared to AA (Table 4). Additionally, moisture content was significantly higher in AA individuals compared to AG or GG. Growth hormone is a master metabolic regulator, which exerts its influence through binding to growth hormone receptors. These findings suggest that the overall balance of muscle synthesis through regulation of fat and protein metabolism is potentially influenced in a complex manner by variation at or nearby the GHR gene (Table 4). Such genotype information has potential for incorporation into management systems for quality.

3.4. SCD

SCD is a protein complex that is key in the synthesis of monounsaturated fatty acids (MUFA). SCD enzyme activity has been shown to correlate with fatty acid composition in a survey of bovine adipose tissue (Yang, Larson, Smith, & Tume, 1999). The A allele at a SNP at position 702 in the open reading frame of the cDNA, was found to contribute to higher MUFA percentage and lower melting point in IMF in muscle of Japanese Black cattle (Taniguchi et al., 2004). Here, this SNP was found to be associated with IMF level ($p<0.05$) and Tukey–Kramer analysis revealed that AA genotypes had significantly higher IMF compared with AG, though GG were not significantly different to either AA or AG (Table 4). This indicates the gene may influence total fat content as well as fatty acid profile in bovine muscle. It was not possible to test if the genotype had effects on MUFA % as this phenotype was not measured for this study. GLM analysis also revealed an association with several colour parameters in LTL and SM muscles, and Tukey–Kramer analysis showed that AA individuals produced lighter beef (higher L^* values) compared with GG, and AA were also less red and more green (lower a^* values), which suggests a

potential link between IMF percentage and colour, perhaps simply because of the higher proportion of white marbling in the tissue but potentially also because MUFA content influences susceptibility to oxidation. This marker may be useful to producers in determining propensity to fat deposition, even in non-Wagyu breeds.

3.5. C/EBP-α

CCAAT/enhancer binding protein (C/EBP-α) is a transcription factor which binds to the leptin promoter and plays an important role in lipid deposition and adipocyte differentiation. A SNP has been identified at position 271 (A/C substitution) of the coding region and animals with AA genotypes displayed higher marbling than those with the AC or CC genotype (Shin et al., 2007). In this study, GLM analysis revealed no association between the C/EBP-α SNP and bovine intra-muscular fat level or other meat quality traits. This concurs with findings of Barendse et al. (2009), wherein association with IMF could not be confirmed definitively in Australian *B. taurus* or *B. indicus*, though a tendency towards association in *B. taurus* was observed. It is possible that the causative SNP may be limited to or rare outside the Hanwoo breed, or the LD relationship between this marker SNP and the functional mutation may differ across populations and thus is not relevant to IMF level in the present population.

3.6. Carboxypeptidase E (CPE)

CPE is a peripheral membrane protein that specifically binds regulated secretory pathway proteins, including prohormones and has been linked to obesity in mice and type 2 diabetes mellitus in the human (Chen et al., 2001). It also maps nearby to a QTL on chromosome 18 for marbling (Barendse et al., 2009). For these reasons, CPE is hypothesised to be a candidate gene for meat quality traits related to IMF level and glucose metabolism in the bovine (Shin & Chung, 2007). Associations with IMF have previously been identified in relation to SNP in this gene in *B. taurus* Hanwoo breed (Shin & Chung, 2007) and in Australian *B. taurus* populations (Barendse et al., 2009). A *Dde*I RFLP in intron 4 identified by Haegeman et al. (2003) was studied here. While the minor allele frequency observed here (0.21) was similar to the observations in Haegeman et al. (2003), GLM analysis indicated that there was no association between this SNP and intra-muscular fat level in the present population. However, a significant association with flavour in LTL was observed. Larger sample sizes may be required to detect a significant effect on IMF in the present sample or this SNP may not be associated with the trait in this genetic background. In relation to the role of the gene in glucose metabolism, we observed a correlation between SNP allele frequencies and cook loss which may point to a link with the anaerobic metabolic pathways that contribute to the rate of postmortem glycolysis, pH decline and protein denaturation (Thompson et al., 2006).

Overall, for the SNP under investigation in this study, we have, in several cases confirmed an association with quality. In other cases we have extended association with traits to new species e.g. the association between a PRKAG3 SNP and cooking loss in beef and between CAST and ultimate pH. However, in this study, associations between CAST and Warner–Bratzler shear force or CPE and intra-muscular fat were not confirmed. Studies in pork in which SNP in candidate genes for quality have been assessed in multiple populations simultaneously (Wimmers et al., 2007) have also identified a lack of consistency of association among unrelated populations; associations between SNP and particular traits were only identified in one or a subset of populations, though a lack of high power reflecting smaller sample sizes may also play a role in the present study. However, there is consensus that few significant associations with meat quality traits have been validated in all studied populations, as discussed by Barendse et al. (2009). Generally speaking, where marker SNP do not themselves affect gene expression or function, and the association with traits of interest are due to LD relationships, associations can vary across populations (Andersson, 2001). For this reason as highlighted by Van Eenennaam et al. (2007), assessment in multiple populations is important, before markers are recommended for inclusion in genome-assisted selection programmes or meat quality management programmes.

4. Conclusion

Several associations between analysed SNP genotype frequencies and colour, water-holding capacity and composition traits were identified, though some published associations could not be confirmed. SNP in CAST, PRKAG3, GHR and SCD genes were significantly associated with colour traits. The PRKAG3 SNP was also associated with cook loss in LTL and had a suggestive association with cook loss in SM. Allele frequencies in CAST and SCD genes were correlated with ultimate pH values. A SNP in the GHR gene was influential on muscle composition i.e. intra-muscular fat, protein and moisture content. These SNP may have predictive value in relation to technological properties of beef, however few associations with sensory quality scores were observed. This is the first time some genes have been linked to particular meat quality traits (e.g. CPE and water-holding capacity in beef) and further investigation of novel associations in independent populations is recommended.

Acknowledgements

This research was funded by Irish Government National Development Plan under the Food Institutional Research Measure (FIRM). We wish to thank Paula Reid and Liselotte Pannier of Ashtown Food Research Centre for their assistance with sampling and statistical analysis and Ozlem Aslan for helpful comments on the manuscript.

References

Adamowicz, T., Pers, E., & Lechniak, D. (2005). A new SNP in the 3 ′-UTR of the hsp 70-1 gene in *Bos taurus* and *Bos indicus*. *Biochemical Genetics*, *43*(11–12), 623−627.
Andersson, L. (2001). Genetic dissection of phenotypic diversity in farm animals. *Nature Reviews Genetics*, *2*, 130−138.
Barendse, W. J. (2009). *DNA markers for meat tenderness*. United States Patent 7625698. http://www.freepatentsonline.com/7625698.html
Barendse, W., Bunch, R. J., & Harrison, B. E. (2009). Variation at CPE but not CEBPA appears to be associated with intramuscular fat deposition in the *longissimus* muscle of cattle. *Animal Production Science*, *49*(7), 558−562.
Barendse, W., Harrison, B. E., Hawken, R. J., Ferguson, D. M., Thompson, J. M., Thomas, M. B., et al. (2007). Epistasis between calpain 1 and its inhibitor calpastatin within breeds of cattle. *Genetics*, *176*, 2601−2610.
Bernard, C., Cassar-Malek, I., Le Cunff, M., Dubroeucq, H., Renand, G., & Hocquette, J. F. (2007). New indicators of beef sensory quality revealed by expression of specific genes. *Journal of Agricultural and Food Chemistry*, *55*(13), 5229−5237.
Casas, E., White, S., Wheeler, T. L., Shackelford, S. D., Koohmaraie, M., Riley, D., et al. (2006). Effects of calpastatin and mu-calpain markers in beef cattle on tenderness traits. *Journal of Animal Science*, *84*, 520−525.
Chen, H., Jawahar, S., Qian, Y. M., Duong, Q. Y., Chan, G. Y., Parker, A., et al. (2001). Missense polymorphism in the human carboxypeptidase E gene alters enzymatic activity. *Human Mutation*, *18*(2), 120−131.
Ciani, E., Roux, M., Ciampolini, R., Mazzanti, E., Cecchi, F., Tancredi, M., et al. (2007). Haplotype association analysis of meat quality traits at the bovine PRKAG3 locus. *Italian Journal of Animal Science*, *6*, 82−84.
Ciobanu, D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G., et al. (2001). Evidence for new alleles in the protein kinase monophospate-activated y3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. *Genetics*, *159*, 1151−1162.
Ciobanu, D. C., Bastiaansen, J. W. M., Lonergan, S. M., Thomsen, H., Dekkers, J. C. M., Plastow, G. S., et al. (2004). New alleles in calpastatin gene are associated with meat quality traits in pigs. *Journal of Animal Science*, *82*(10), 2829−2839.
Costello, S., O'Doherty, E., Troy, D. J., Ernst, C. W., Kim, K. S., Stapleton, P., et al. (2007). Association of polymorphisms in the calpain I, calpain II and growth hormone genes with tenderness in bovine *M longissimus dorsi*. *Meat Science*, *75*(4), 551−557.
Di Stasio, L., Brugiapaglia, A., Destefanis, G., Albera, A., & Sartore, S. (2003). GH1 as candidate gene for variability of meat production traits in Piemontese cattle. *Journal of Animal Breeding and Genetics*, *120*, 358−361.
Di Stasio, L., Destefanis, G., Brugiapaglia, A., Albera, A., & Rolando, A. (2005). Polymorphism of the GHR gene in cattle and relationships with meat production and quality. *Animal Genetics*, *36*(2), 138−140.
Dikeman, M. E., Pollak, E. J., Zhang, Z., Moser, D. W., Gill, C. A., & Dressler, E. A. (2005). Phenotypic ranges and relationships among carcass and meat palatability traits for fourteen cattle breeds, and heritabilities and expected progeny differences for

Warner–Bratzler shear force in three beef cattle breeds. *Journal of Animal Science*, *83*(10), 2461−2467.
Grosz, M. D., Skow, L. C., & Stone, R. T. (1994). An *AluI* polymorphism at the bovine 70 kD heat shock protein-1 (HSP70-1) locus. *Animal Genetics*, *25*(3), 196.
Haegeman, A., Williams, J. L., Law, A., Van Zeveren, A., & Peelman, L. J. (2003). Mapping and SNP analysis of bovine candidate genes for meat and carcass quality. *Animal Genetics*, *34*, 349−353.
Han, S. H., Cho, I. C., Kim, J. H., Ko, M. S., Jeong, H. Y., Oh, H. S., et al. (2009). A GHR polymorphism and its associations with carcass traits in Hanwoo cattle. *Genes & Genomics*, *31*(1), 35−41.
Hochberg, Y. (1988). A sharper Bonferroni procedure for multiple tests of significance. *Biometrika*, *43*, 800−802.
Hoj, S., Fredholm, M., Larsen, N. J., & Nielsen, V. H. (1993). Growth hormone gene polymorphism associated with selection for milk-fat production in lines of cattle. *Animal Genetics*, *24*(2), 91−95.
Joo, S., Kauffman, R., Kim, B., & Kim, C. (1995). The relationship between color and water-holding capacity in postrigor porcine *longissimus* muscle. *Journal of Muscle Foods*, *6*(3), 211−226.
Koohmaraie, M., Whipple, G., Kretchmar, D. H., Crouse, J. D., & Mersmann, H. J. (1991). Postmortem proteolysis in *Longissimus* muscle from beef, lamb and pork carcasses. *Journal of Animal Science*, *69*(2), 617−624.
Kristensen, L., & Purslow, P. P. (2001). The effect of ageing on the water-holding capacity of pork: Role of cytoskeletal proteins. *Meat Science*, *58*(1), 17−23.
Maher, S. C., Mullen, A. M., Moloney, A. P., Buckley, D. J., & Kerry, J. P. (2004). Quantifying the extent of variation in the eating quality traits of the *M longissimus dorsi* and *M semimembranosus* of conventionally processed Irish beef. *Meat Science*, *66*(2), 351−360.
Malek, M., Dekkers, J. C. M., Lee, H. K., Baas, T. J., Prusa, K., Huff-Lonergan, E., et al. (2001). A molecular genome scan analysis to identify chromosomal regions influencing economic traits in the pig. II. Meat and muscle composition. *Mammalian Genome*, *12*(8), 637−645.
McKay, S. D., White, S. N., Kata, S. R., Loan, R., & Womack, J. E. (2003). The bovine 5′ AMPK gene family: mapping and single nucleotide polymorphism detection. *Mammalian Genome*, *14*(12), 853−858.
Melody, J. L., Lonergan, S. M., Rowe, L. J., Huiatt, T. W., Mayes, M. S., & Huff-Lonergan, E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. *Journal of Animal Science*, *82*(4), 1195−1205.
Milan, D., Jeon, J. T., Looft, C., Amarger, V., Robic, A., Thelander, M., et al. (2000). A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science*, *288*(5469), 1248−1251.
Offer, G., & Knight, P. (1988). Drip losses. In R. Lawrie (Ed.), *Developments in meat science*. London, UK: Elsevier.
Pannier, L., Sweeney, T., Hamill, R. M., Ipek, F., Stapleton, P. C., & Mullen, A. M. (2009). Lack of an association between single nucleotide polymorphisms in the bovine leptin gene and intramuscular fat in *Bos taurus* cattle. *Meat Science*, *81*(4), 731−737.
Raymond, M., & Rousset, F. (1995). GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, *86*, 248−249.
Roux, M., Nizou, A., Forestier, L., Ouali, A., Leveziel, H., & Amarger, V. (2006). Characterization of the bovine PRKAG3 gene: Structure, polymorphism, and alternative transcripts. *Mammalian Genome*, *17*(1), 83−92.
Sawyer, J. T., Apple, J. K., Johnson, Z. B., Baublits, R. T., & Yancey, J. W. S. (2009). Fresh and cooked color of dark-cutting beef can be altered by post-rigor enhancement with lactic acid. *Meat Science*, *83*(2), 263−270.
Schenkel, F., Miller, S., Jiang, Z., Mandell, I., Ye, X., Li, H., et al. (2006). Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science*, *84*, 291−299.
Sentandreu, M. A., Coulis, G., & Ouali, A. (2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in Food Science and Technology*, *13*, 400−421.
Shin, S. C., & Chung, E. R. (2007). SNP detection of carboxypeptidase E gene and its association with meat quality and carcass traits in Korean cattle. *Asian-Australasian Journal of Animal Sciences*, *20*(3), 328−333.
Shin, S. C., Kang, M. J., & Chung, E. R. (2007). Identification of a novel SNP associated with meat quality in C/EBPα gene of Korean cattle. *Asian-Australasian Journal of Animal Sciences*, *4*, 466−470.
Taniguchi, M., Utsugi, T., Oyama, K., Mannen, H., Kobayashi, M., Tanabe, Y., et al. (2004). Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. *Mammalian Genome*, *15*(2), 142−148.
Tatsuda, K., Oka, A., Iwamoto, E., Kuroda, Y., Takeshita, H., Kataoka, H., et al. (2008). Relationship of the bovine growth hormone gene to carcass traits in Japanese black cattle. *Journal of Animal Breeding and Genetics*, *125*(1), 45−49.
Thompson, J. M., Perry, D., Daly, B., Gardner, G. E., Johnston, D. J., & Pethick, D. W. (2006). Genetic and environmental effects on the muscle structure response post-mortem. *Meat Science*, *74*(1), 59−65.
Van Eenennaam, A. L., Li, J., Thallman, R. M., Quaas, R. L., Dikeman, M. E., Gill, C. A., et al. (2007). Validation of commercial DNA tests for quantitative beef quality traits. *Journal of Animal Science*, *85*(4), 891−900.
Viitala, S., Szyda, J., Blott, S., Schulman, N., Lidauer, M., Maki-Tanila, A., et al. (2006). The role of the bovine growth hormone receptor and prolactin receptor genes in milk, fat and protein production in Finnish Ayrshire dairy cattle. *Genetics*, *173*(4), 2151−2164.
Wheeler, T. L., Cundiff, L. V., Shackelford, S. D., & Koohmaraie, M. (2004). Characterization of biological types of cattle (Cycle VI): Carcass, yield, and *longissimus* palatability traits. *Journal of Animal Science*, *82*(4), 1177−1189.
Wheeler, T., Shackleford, S., & Koohmaraie, M. (1996). Sampling, cooking, and coring effects on Warner–Bratzler shear force values in beef. *Journal of Animal Science*, *74*(7), 1553−1562.
Wimmers, K., Murani, E., Pas, M., Chang, K. C., Davoli, R., Merks, J. W. M., et al. (2007). Associations of functional candidate genes derived from gene-expression profiles of prenatal porcine muscle tissue with meat quality and muscle deposition. *Animal Genetics*, *38*(5), 474−484.
Yang, A., Larson, T., Smith, S., & Tume, R. (1999). Δ9 desaturase activity in bovine subcutaneous adipose tissue of different fatty acid composition. *Lipids*, *34*, 971−978.
Yu, S. L., Kim, J. E., Chung, H. J., Jung, K. C., Lee, Y. J., Yoon, D. H., et al. (2005). Molecular cloning and characterization of bovine PRKAG3 gene: Structure, expression and single nucleotide polymorphism detection. *Journal of Animal Breeding and Genetics*, *122*(5), 294−301.