Associations between single nucleotide polymorphisms in multiple candidate genes and carcass and meat quality traits in a commercial Angus-cross population

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ABSTRACT

The aim of this study was to evaluate the effects of 28 single nucleotide polymorphisms (SNP) in 10 candidate genes previously shown to be associated with quality traits in pigs and cattle. The data set comprised 28 traits recorded on a commercial population of 536 Aberdeen Angus-cross beef cattle. Among the traits, 20 were carcass and sirloin quality related, one mechanical measure of tenderness, and the remaining seven were taste panel assessed sensory traits. The candidate genes studied included growth hormone (GH) and pro-opiomelanocortin (POMC). Association analysis showed that 13 of the 28 SNPs were significantly associated with at least one of the traits. Some of these were novel (POMC and mechanical tenderness), whilst others confirmed previous results (GH and eye muscle length).
Following validation in other populations and breeds, these markers could be incorporated into breeding programs to increase the rate of improvement in carcass and meat quality traits.

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1. Introduction

Meat quality is of great importance to the beef industry where the consumer is willing to pay more for superior products (Dekkers & Hospital, 2002; Shackelford et al., 2001). Traditionally trait improvement in livestock has used quantitative genetics theory to determine animals with high genetic merit (Dekkers & Hospital, 2002). This selection approach is most effectively implemented for highly heritable traits that are easily measured.

Meat quality traits, however, can usually only be measured post-slaughter and often have low heritabilities (Gill et al., 2010; Marshall, 1999) so that making progress using direct measurement is difficult. Marker assisted selection has the potential to increase the rate of genetic improvement. Markers found in various candidate genes linked to economically relevant traits have been identified and incorporated into commercially available genetic tests for meat quality. Whilst it is likely that genomic predictions of genetic merit using dense SNPs will become available for some meat quality traits in the foreseeable future, there are likely to be many breed–trait combinations for which such predictors will not be derived. Hence gene-based tests are likely to remain important.

Markers have been reported in candidate genes that are associated with meat quality traits in pigs and cattle (Buchanan, Thue, Yu, & Winkelman-Sim, 2005; Franco, Antunes, Silva, & Goulart, 2005; Houston, Cameron, & Rance, 2004). However, before such information

can be used in breeding programs it is important that unbiased and independent validation studies in different breeds are carried out to establish whether the observed effects are found in the breeds or populations of interest.

The present study tested associations between 28 single nucleotide polymorphisms (SNP) in 10 genes and meat quality traits in commercial animals. The following genes, which tended to be involved with lipid control, appetite control or growth control, were studied: corticotropin releasing hormone (CRH), growth hormone (GH), melanocortin 4 receptor (MC4R), POU class 1 homeobox 1 (POU1F1), pro-opiomelanocortin (POMC), peroxisome proliferator-activated receptor alpha (PPARA), protein kinase, AMP-activated, gamma 3 non-catalytic subunit (PRKAG3), somatostatin (SST), phosphoglycerate mutase 2 (muscle) (PGAM2) and insulin-like growth factor 2 (IGF2). CRH has been associated with subcutaneous fat depth and beef marbling score in cattle (Wibowo, Michal, & Jiang, 2007). GH has been associated with marbling and rump fat in feedlot cattle (Barendse, Bunch, Harrison, & Thomas, 2006). MC4R has been shown to be associated with ultrasonic backfat depth, average daily gain and daily feed intake in pigs (Houston et al., 2004). POU1F1 has been associated with fat thickness (Franco et al., 2005), backfat depth and birth weight (Yu, Tuggle, Schmitz, & Rothschild, 1995). POMC has been associated with shipping and hot carcass weight (Buchanan et al., 2005). PPARA is located close to a porcine QTL for backfat (Szczerbal et al., 2007). PRKAG3 has been associated with water holding capacity and cooking loss in pigs (Ciobanu et al., 2001; Lindahl et al., 2004). SST has been associated with marbling score and yearling height (Morsci, Schnabel, & Taylor, 2006). PGAM2 has been associated with drip loss in pigs (Fontanesi, Davoli, Costa, Scotti, & Russo, 2003).

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IGF2 has been associated with longissimus muscle area, fat % (Sherman et al., 2008) and rib-eye area (Goodall & Schmutz, 2007).

2. Materials and methods

2.1. Sample collection

Commercial crossbred beef cattle (n = 536 animals) with purebred Aberdeen Angus sires were sourced through the Scotbeef abattoir (Bridge of Allan, Scotland). Cattle originated from 16 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, being a mix of heifers and steers ranging between 406 and 913 days old at kill, with the age differences depending largely on the farm. The 536 animals used in the experiment included 164 females and 372 males. Carcass weight ranged from 213 to 475 kg with an average of 324 kg. The sires for all animals were purebred Aberdeen Angus whilst the dams were a mixture of purebreds of various breeds and crossbreeds, including Aberdeen Angus, Aberdeen Angus-cross, Simmental-cross and Limousin-cross.

Cattle were stunned by captive bolt before being slaughtered by exsanguination and dressed using standard commercial specifications. During exsanguination, 100 mL blood was collected and frozen for DNA extraction.

2.2. Carcass trait measurement

At slaughter, hot carcass weight was recorded and carcasses were graded by a Meat Hygiene Service assessor for muscle composition and carcass fatness according to the standard European Union beef carcass classification scale (EUROP) (Hickey, Keane, Kenny, Cromie, & Veerkamp, 2007). Conformation and fat class scores were transformed into a 7-point numerical scale (Kempster, Cook, & Grantley-Smith, 1986). All other measurements were made by workers at the Scotbeef processing plants. Twenty-four hours after slaughter, pH and temperature were recorded in the sirloin muscle with the TESTO 205 pH meter (TESTO, Hampshire, UK) and ETI FPT thermometer (ETI Ltd. Worthing, UK), respectively.

At deboning, weight of the hindquarter and sirloins were recorded. Sirloins were vacuum-packed and stored below 4 °C for 21–30 days to mature, then removed from the vacuum pack, patted dry to remove excess moisture and weighed. Three steaks were cut from the centre of the sirloin as follows: for tenderometer testing 3–4 cm thick, for sirloin measurements 1–2 cm thick and for sensory testing 2 cm thick.

For tenderometer testing, steaks were trimmed to 200–220 g of eye muscle and placed in a water bath at 100 °C until the centre of the sample reached 82 °C. Samples were left to cool to 7 °C then tested using a MIRINZ Tenderometer machine (AgResearch, Hamilton, New Zealand) which measures toughness using kPa (equivalent to kN/m^2). Seven traits were measured on the sirloin steak: sirloin fat depth, eye muscle length, eye muscle depth, tail length (i.e. the length of the area of fat attached to the side of the sirloin steak), gristle distance from eye muscle base and gristle distance from fat band.

These were then used to calculate a number of additional traits as follows:

1. Eye length as a percentage of sirloin steak length = 100 × (Eye muscle length/(Eye muscle length + Tail length))
2. Eye area = Eye muscle length × Eye muscle depth
3. Gristle encroachment = 100 × (Gristle distance from fat band/ (Gristle distance from fat band + Gristle distance from eye muscle base)).

A full list of analysed carcass quality traits can be found in Table 1.

Table 1
Number of carcass trait observations, means and coefficients of variation.

Trait	n	Mean	CV
Tenderometer score, kPa[a]	482	24.42	21.13
Hot carcass weight, kg	536	324.22	12.38
Sirloin weight before maturation, kg	472	7.22	14.39
Sirloin weight after maturation, kg	481	7.16	14.5
Conformation class, transformed numerical scale	536	7.22	21.26
Cooking loss, g	475	65.01	14.05
Eye muscle length as a % of sirloin length	476	77.65	10.7
Eye muscle area, mm²	481	11020	15.39
Eye muscle depth, mm	481	70.02	12.18
Eye muscle length, mm	481	157.26	8.46
Fat class, transformed numerical scale	536	8.59	12.08
Sirloin fat depth, mm	472	6.47	52.93
Gristle encroachment, mm	462	20.44	42.33
Gristle distance from eye muscle base, mm	481	53.99	20.84
Gristle distance from fat band, mm	462	14.04	44.95
Gristle length, mm	481	70.45	21.95
Sirloin weight as % of hindquarter weight	462	9.71	10.28
Sirloin steak tail length, mm	475	46.74	42.53
Temperature at 24 h, °C	516	4.08	13.12
Hindquarter weight, kg	498	74.2	11.41
pH at 24 h	458	5.55	2.39

[a] Unit of measurement for Tenderometer was kilopascals (equivalent to kN/m^2).

2.3. Taste panel selection and assessments

Taste panel members were chosen among workers at the Scotbeef meat processing plant in East Kilbride, Scotland. Members of staff (n = 38) were tested using the Triangle and Matching tests (BSI-BS7667, 1993) with 10 being discarded due to poor scores. Taste panels included six members and an average of nine samples were tested in one sitting with the addition of one blind repeat steak per panel. Participants were instructed to rinse their mouths with water before tasting began as well as between samples. They were also instructed not to eat or drink for 1 h prior to the test.

Prior to assessment sirloin steaks were cooked using a Lincat Lynx 400 electric griddle (Lincat Ltd, Lincoln, UK) until a thermometer placed in the centre of the steak reached 74 °C. The six panelists then scored the steaks on a 1–8 scale for seven sensory traits, a full list of which can be seen in Table 2 along with an explanation of the scoring scheme used.

2.4. SNP genotyping

Samples were genotyped at 28 SNPs from 10 different genes by KBioscience Ltd using a fluorescence-based competitive allele specific PCR (KASPar) assay (http://www.kbioscience.co.uk). The SNP locations in each gene, Genbank accession number and the positions (intron/exon etc) are listed in Supplementary Table 1. All animals with phenotypes were genotyped, as were all available sires (42).

2.5. Data analysis

2.5.1. SNP frequencies and linkage disequilibrium

Genotype frequencies of each polymorphism were tested for deviations from Hardy–Weinberg equilibrium by χ^2 tests (Falconer &

Table 2
Number of observations, means and coefficients of variation for sensory traits measured by the taste panel.

Trait	1	8	n	Mean	CV
Abnormal flavour	Extremely strong	Extremely weak	521	6.29	12.72
Abnormal odour	Extremely strong	Extremely weak	521	6.41	11.81
Flavour	Extremely weak	Extremely strong	521	5.51	9.90
Odour	Extremely weak	Extremely strong	521	5.26	10.23
Juiciness	Extremely dry	Extremely juicy	521	5.55	12.32
Tenderness	Extremely tough	Extremely tender	521	5.75	11.33
Overall liking	Disliked extremely	Liked extremely	521	5.63	10.93

Mackay, 1997). Pairwise genotype combinations of the SNPs in the same gene were also tested for linkage disequilibrium (LD), the degree of non-random association of alleles at two or more loci, using the Haploview program, version 4 (Barrett, Fry, Maller, & Daly, 2005). The Haploview program uses a two-marker EM (expectation maximisation) to estimate the maximum-likelihood values of the four gamete frequencies and also calculates D′, r² values and linkage LOD scores, where D′ is the normalized covariance for a given SNP pair, r² is the squared correlation coefficient between the two SNPs and LOD is the log of the likelihood odds ratio, a measure of confidence in the value of D′.

2.5.2. Mixed model association analysis

The relationship between the different genotypes of each SNP and the various traits recorded was evaluated using a single marker mixed model association analysis. Data were analysed 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) and interaction of sire and slaughter date (panel date for the taste panel traits). An additional term including animal ID and steak ID (A or B) was added for analysis of taste panel traits to allow a distinction to be made between the A and B steaks of those animals that had repeat steaks tested. The general model used for carcass traits was as follows:

$$Y_{ijklmno} = \mu + F_j + G_k + S_l + (G \times S)_{kl} + K_m + M_{jn} + e_{ijklmno}$$

where:

Y_{ijklmn}	is the trait measured on the individual i
μ	is the overall mean for the trait
F_j	is the fixed effect of farm j (14 levels)
G_k	is the fixed effect of SNP genotype k (3 levels)
S_l	is the fixed effect of sex l (2 levels)
$(G \times S)_{kl}$	is the interaction between the k-th SNP genotype and the l-th sex
K_m	is the random effect of kill-date m
M_{jn}	is the random effect of the n-th sire on the j-th farm
e_{jklmno}	is the residual term associated with the observation.

Additional interaction terms between sire and kill-date were fitted as random effects. Variance components were constrained to be non-negative, i.e. where effects were estimated to be negative they were set to zero.

For the taste panel traits the general model was as follows:

$$Y_{ijklmnop} = \mu + F_j + G_k + S_l + (G \times S)_{kl} + P_m + M_{jn} + T_{io} + e_{ijklmnop}$$

where additional terms are:

P_m	is the random effect of taste panel date m
T_{io}	is the random effect of the o-th steak (A or B) for the i-th animal
$e_{ijklmnop}$	is the residual error associated with the observation.

Again, additional interaction terms between sire and taste panel date were fitted as random effects, and variance components were constrained to be non-negative.

The effects of several covariates (percentage Aberdeen Angus, hot carcass weight and age at kill) were also examined in separate analyses. The percentage Aberdeen Angus (% AA) was based on dam breed, as all bulls were purebred Aberdeen Angus, so that each animal was assigned a value of 100% (if the dam was AA), 75% (if the dam was AA-cross) or 50% (if the dam was neither). Each covariate was tested

individually. Statistical significance for the fixed effects was determined using approximate F-statistics with denominator degrees of freedom (Kenward & Roger, 1997) estimated in the Genstat REML procedure.

Additive effects and dominance deviations were also calculated using a re-parameterized model. The additive effect was estimated as the difference between the mean of the two homozygotes divided by two, and dominance was estimated as the deviation of the heterozygote from the mean of the two homozygotes (Falconer & Mackay, 1997). For some SNPs only two genotype classes (the heterozygote and one homozygote) were present, therefore, it was not possible to separate the additive and dominance effects of the gene.

2.5.3. Multiple SNP analysis

In order to test the effects of multiple SNPs within a single gene, two approaches were adopted. First the observed genotype effect, in cases where more than one SNP from the same gene was associated with a single trait, was investigated. To do this an analysis was carried out including multi-locus SNP genotypes (two SNPs in all cases) in the fixed model. Whilst the random model remained unchanged the fixed model became: farm + sex + SNP1 + SNP2 + SNP1 · SNP2, where the last term represents the interaction between the two SNPs.

Secondly a haplotype based approach was used. Haplotypes were reconstructed for all genes that contained more than one SNP using software that determines the gametic haplotypes for each animal where phase is known with certainty based on sire and sibling genotype information (Pong-Wong, George, Woolliams, & Haley, 2001). Haplotype pairs (sire and dam) were unambiguously reconstructed for the CRH gene (215 individuals), the GH gene (321), the MC4R gene (411), the PGAM2 gene (290), the POU1F1 gene (326), the POMC gene (258), the PPARA gene (298) and the PRKAG3 gene (242) out of the 536 genotyped animals.

In order to determine whether the haplotype information accounted for additional variation beyond the SNP genotype analysis, the haplotype group (a combination of the two haplotypes) was nested within a SNP model i.e. the model was the same as the genotype model but with additional fixed terms accounting for the variation between the haplotype groups within the SNP genotype groups in the fixed model. This analysis was carried out for each of the traits found to be significantly affected by any of the SNPs in the candidate genes. Statistical significance of the extra variation accounted for by the presence of the haplotype groups in the model was determined using approximate F-statistics derived from Wald statistics with denominator degrees of freedom estimated in the Genstat REML procedure (Kenward & Roger, 1997).

2.5.4. Correction for multiple testing

To correct for the large number of traits analysed with a large number of SNPs, and hence a high probability of false positive results, a Bonferroni correction was applied. The majority of SNPs in each gene were found to be in partial or strong LD so that the effective number of SNPs tested was estimated as 10 (the number of genes tested). The correction for multiple SNP testing resulted in an adjusted P value of 0.005 for the 5% significance level.

3. Results

3.1. Phenotypic variation

A total of 28 phenotypic measurements were recorded on 536 animals. These traits included seven taste panel assessed sensory traits, one mechanical measure of tenderness and 20 carcass and sirloin measurements. The number of records, trait means and coefficients of variation are given in Tables 1 and 2.

3.2. Genotypic frequencies

All 536 animals were genotyped at the 28 SNPs (successful assignment was possible for 97% of genotypes). Some SNPs were fixed in the population studied: *MC4R A530G*, *PGAM2 C272T* and *PRKAG3 T172G*, where the A, C and G alleles respectively were fixed. All but two of the 28 SNPs were in Hardy–Weinberg equilibrium (Falconer & Mackay, 1997). Those not in H–W equilibrium were *CRH C22G* and *POMC C254T* where there was an excess of animals with the CG and CC genotypes respectively. SNPs in the same gene were tested for linkage disequilibrium (LD) (Table 3). In most cases D′ values were high but r^2 values tended to be less than 0.5 between the majority of same-gene SNP pairs. However, four within-gene SNP pairs had high r^2 values. These were the *POU1F1* SNPs, *T606C* and *A647G*, the *POMC* SNPs, *C674T* and *C783T*, the *PRKAG3* SNPs, *A2961G* and *A5534G* and the *GH* SNPs, *A556C* and *C296T* where the r^2 values were 0.98, 0.92, 1 and 1 respectively.

3.3. Genotype effects

SNPs in eight of the genes tested showed significant associations with 14 of the traits tested, resulting in 20 significant SNP-trait associations (Table 4). SNPs in the *PGAM2* and *IGF2* genes were not associated with any of the traits tested.

In general associations were with weight-related carcass quality traits or those measured on the sirloin steaks, only one association was with a taste panel assessed sensory trait: this was the association between one of the *PPARA* SNPs and juiciness (P = 0.04). Multiple SNPs in different genes were associated with eye muscle length, specifically, two of the *GH* SNPs (*A556C* and *C296T*) (P = 0.04 for both) and one of the *POMC* SNPs (*C254T*) (P = 0.02) and with eye muscle area and eye muscle length as a percentage of sirloin steak length. Two other *POMC* SNPs (*C674T* and *C783T*) that are in strong LD with each other were associated with tenderometer score where, for both SNPs, animals with the CC genotype were found to have lower scores. Additionally, these two SNPs were significantly associated with taste panel assessed tenderness, although not in an additive manner (in both cases animals with the CT genotype were found to be the most

tender). Furthermore, there were associations observed between sirloin fat depth and sirloin weight after maturation with a *PPARA* SNP, between gristle related traits and *CRH*, *POU1F1* and *PRKAG3* SNPs, between pH at 24 h and both *CRH* and *PPARA* SNPs, and between fat class and a *MC4R* SNP (Table 4). Correction for multiple testing resulted in an adjusted P value of 0.005 for the 5% significance level so that only the associations between *CRH A145G* and both pH and gristle distance from fat band remain significant (Table 3).

The degree of dominance was only significant for a few associations. Those that were significant included the *CRH A145G* SNP effect on pH at 24 h, the *PIT A674G* SNP effect on gristle length and both *POMC* SNPs on tenderness (Table 4).

To further investigate the observed associations and correct the data for potential biases, a number of covariates were added to the model (one at a time). These included hot carcass weight, age at kill and percentage Aberdeen Angus (50, 75 or 100%, depending on dam breed). Introducing hot carcass weight into the model increased the P value of associations seen between the *POMC C254T* SNP and the eye muscle-related traits, the *PPARA A82G* SNP and sirloin weight after maturation and also the *SST A447G* SNP and eye muscle depth so that the genotype effects were no longer significant.

The only SNP-trait association significantly affected by % AA was the association between *PRKAG3 C2180T* and hot carcass weight where an increase in % AA led to a decrease in hot carcass weight. Incorporating this covariate into the model reduced the significance level of the SNP-trait association. Age at kill made no difference to the genotypic effects for any of the significant traits, possibly because most of the age differences between animals are already accounted for in the model as farm effects.

3.4. Sex × genotype interactions

There were significant genotype-by-sex interactions for five of the 20 significant trait–SNP genotype associations (Table 5). These were the association between *CRH A145G* and pH after 24 h, the association between *MC4R C1069G* and fat class, the association between *POMC C254T* and eye muscle length, the association between *PPARA A82G* and sirloin weight after maturation and the association between *PPARA*

Table 3
Haplotypes frequencies for genes with more than one segregating SNP.

Gene	Haplotype	Frequency	r^2 (D′) values			
CRH[a]	AGG	0.195		*A145G*	*C22G*	
	GCC	0.205	*C22G*	0.27 (1)		
	GCG	0.279	*C240G*	0.06 (1)	0.26 (1)	
	GGG	0.316				
GH[b]	GACT	0.044		*G212T*	*A556C*	*C296T*
	GCTT	0.031	*A556C*	0.33 (1)		
	TACC	0.26	*C296T*	0.36 (1)	1 (1)	
	TACT	0.664	*C442T*	0.06 (1)	0.002 (0.3)	0.01 (0.53)
POU1F1[c]	CG	0.248		*T606C*		
	TA	0.752	*A647G*	0.98 (1)		
POMC[d]	CCC	0.591		*C254T*	*C674T*	
	CTT	0.337	*C674T*	0.21 (1)		
	TTT	0.064	*C783T*	0.17 (1)	0.92 (1)	
PPARA[e]	CA	0.062		*C232G*		
	GA	0.604	*A82G*	0.07 (1)		
	GG	0.332				
PRKAG3[f]	TCAAGG	0.227		*C2180T*	*A2961G*	*A3078G*	*C3163G*
	TTAAGG	0.167	*A2961G*	0.22 (1)			
	TTAGGG	0.143	*A3078G*	0.42 (1)	0.52 (1)		
	TTGGCG	0.461	*C3163G*	0.22 (1)	1 (1)	0.52 (1)	
			A5534G	0.01 (1)	0.01 (1)	0.01 (1)	0.01 (1)

[a] *CRH* haplotype: *A145G*, *C22G*, and *C240G*.
[b] *GH* haplotype: *G212T*, *A556C*, *C296T*, and *C442T*.
[c] *POU1F1* haplotype: *T606C* and *A647G*.
[d] *POMC* haplotype: *C254T*, *C674T*, and *C783T*.
[e] *PPARA* haplotype: *C232G* and *A82G*.
[f] *PRKAG3* haplotype: *T172G*, *C2180T*, *A2961G*, *A3078G*, *C3163G*, and *A5534G*.

Table 4
Genotype means, standard errors, P values and estimates of additive and dominance effects for SNP with significant trait associations.

Trait	SNP	Genotype means ± se[a]			Overall P value[b]	a ± se[a, c]	d ± se[a, d]
	CRH A145G	AA	AG	GG			
Gristle distance from fat band, mm		18.3 ± 1.42	15.2 ± 0.73	14.0 ± 0.65	0.004[e]	−2.16 ± 0.69**	−0.93 ± 0.85
pH at 24 h		5.65 ± 0.03	5.54 ± 0.01	5.57 ± 0.01	0.002[e]	−0.04 ± 0.02**	−0.07 ± 0.02**
	MC4R C1069G	CC	CG	GG			
Fat class, transformed numerical scale		8.52 ± 0.09	8.77 ± 0.10	8.90 ± 0.17	0.01	0.19 ± 0.09*	0.06 ± 0.11
	POMC C254T	CC	CT	TT			
Eye muscle length as a % of sirloin length		76.7 ± 1.02	77.4 ± 1.28	82.2 ± 2.49	0.05	2.78 ± 1.16*	−2.03 ± 1.44
Eye muscle area, mm²		11,102 ± 163	11,304 ± 234	12,371 ± 508	0.03	635 ± 244**	−432 ± 303
Eye muscle length, mm		156 ± 1.20	159 ± 1.80	165 ± 3.90	0.02	4.43 ± 1.86*	−1.65 ± 2.32
	POMC C674T	CC	CT	TT			
Tenderometer score, kPa[f]		23.3 ± 0.70	24.3 ± 0.66	25.4 ± 0.77	0.01	1.02 ± 0.03**	−0.06 ± 0.42
Tenderness		5.67 ± 0.08	5.84 ± 0.07	5.64 ± 0.09	0.01	−0.02 ± 0.05	0.18 ± 0.06**
	POMC C783T	CC	CT	TT			
Tenderometer score, kPa[f]		23.4 ± 0.76	24.3 ± 0.71	25.1 ± 0.81	0.03	0.85 ± 0.33**	0.10 ± 0.43
Tenderness		5.72 ± 0.09	5.87 ± 0.08	5.68 ± 0.10	0.03	−0.02 ± 0.05	0.17 ± 0.06**
	PPARA A82G	AA	AG	GG			
Sirloin weight after maturation, kg		7.11 ± 0.12	7.18 ± 0.11	7.46 ± 0.15	0.04	0.17 ± 0.07*	−0.10 ± 0.09
Sirloin fat depth, mm		6.07 ± 0.37	6.72 ± 0.35	7.78 ± 0.51	0.01	0.86 ± 0.27**	−0.21 ± 0.33
	PPARA C232G	CC	CG	GG			
pH at 24 h		–	5.53 ± 0.02	5.57 ± 0.01	0.01	–	–
Juiciness		–	5.44 ± 0.10	5.60 ± 0.08	0.04	–	–
	PRKAG3 C2180T	CC	CT	TT			
Hot carcass weight, kg		327 ± 5.80	316 ± 3.60	314 ± 3.50	0.05	−6.63 ± 2.72*	−3.82 ± 3.29
	PRKAG3 A5534G	AA	AG	GG			
Gristle distance from eye muscle base, mm		–	47.1 ± 3.60	53.8 ± 1.30	0.05	–	–
	POU1F1 A647G	AA	AG	GG			
Gristle length, mm		72.8 ± 1.98	74.6 ± 1.98	67.6 ± 2.69	0.01	−2.58 ± 1.12*	4.46 ± 1.44**
	GH A556C	AA	AC	CC			
Eye muscle area, mm²		11,104 ± 154	11,633 ± 290	–	0.05	–	–
Eye muscle length, mm		156 ± 1.20	161 ± 2.20	–	0.04	–	–
	GH C296T	CC	CT	TT			
Eye muscle length as a % of sirloin length		76.9 ± 1.00	79.4 ± 1.47	–	0.01	–	–
Eye muscle length, mm		156 ± 1.20	160 ± 2.10	–	0.04	–	–
	SST A447G	AA	AG	GG			
Eye muscle depth, mm		70.7 ± 0.86	73.3 ± 1.40	–	0.04	–	–

[a] Mean values were adjusted for farm and averaged over sex. Estimate of the effect is expressed in units of the trait.
[b] P value for the overall genotype effect.
[c] Additive effect is estimated as the difference between the 2 homozygous means divided by 2. Where only two genotype classes are present for a SNP the additive effect could not be estimated.
[d] Dominance effect is estimated as the non-additive genetic effects or the deviation of the heterozygote from the mean of the 2 homozygotes. Where only two genotype classes are present for a SNP the dominance effect could not be estimated.
[e] Associations remain significant following correction for multiple testing (adjusted P value was 0.005).
** $P < 0.01$.
* $P < 0.05$.
[f] Units for tenderometer score are kilopascals (equivalent to kN/m²).

C232G and juiciness. Specific results can be seen in Table 5, however, in general these genotype–trait associations were stronger in the female animals; out of the five cases there was only one where there was a significant difference between genotypes in the male animals. Males with the AG genotype at the CRH A145G SNP had significantly higher pH 24 h after slaughter than males with the GG genotype (whilst female animals with the AA genotype at this SNP had significantly higher pH than females with the AG or GG genotypes).

3.5. Multiple SNP analysis

3.5.1. Multiple SNP model

There were two cases where more than one SNP from the same gene affected the same trait, these were: the effect of GH A556C and C296T on eye muscle length and the effect of POMC C674T and C783T on tenderometer score. In both cases the D′ between the SNP in the same gene was 1, whilst the r² value was 1 between the GH SNPs and 0.92

Table 5
Genotype means and standard errors for each genotype-by-sex group.

	Sex	Genotype	Genotype means ± se[c]				
			pH at 24 h	Fat class, transformed numerical scale	Eye muscle length, mm	Sirloin weight after maturation, kg	Juiciness
CRH A145G	F	AA	5.74 ± 0.05[a]	–	–	–	–
		AG	5.56 ± 0.02[b]	–	–	–	–
		GG	5.57 ± 0.02[b]	–	–	–	–
	M	AA	5.57 ± 0.03[a,b]	–	–	–	–
		AG	5.53 ± 0.02[a]	–	–	–	–
		GG	5.57 ± 0.01[b]	–	–	–	–
MC4R C1069G	F	CC	–	8.48 ± 0.12[a]	–	–	–
		CG	–	9.01 ± 0.14[b]	–	–	–
		GG	–	9.23 ± 0.26[b]	–	–	–
	M	CC	–	8.57 ± 0.10[a]	–	–	–
		CG	–	8.53 ± 0.11[a]	–	–	–
		GG	–	8.58 ± 0.21[a]	–	–	–
POMC C254T	F	CC	–	–	150 ± 1.50[a]	–	–
		CT	–	–	155 ± 2.70[a,b]	–	–
		TT	–	–	168 ± 6.50[b]	–	–
	M	CC	–	–	162 ± 1.40[a]	–	–
		CT	–	–	163 ± 1.90[a]	–	–
		TT	–	–	162 ± 3.90[a]	–	–
PPARA A82G	F	AA	–	–	–	6.71 ± 0.15[a]	–
		AG	–	–	–	6.80 ± 0.14[a]	–
		GG	–	–	–	7.43 ± 0.22[b]	–
	M	AA	–	–	–	7.51 ± 0.13[a]	–
		AG	–	–	–	7.56 ± 0.12[a]	–
		GG	–	–	–	7.48 ± 0.16[a]	–
PPARA C232G	F	CC	–	–	–	–	–
		CG	–	–	–	–	5.37 ± 0.14[a]
		GG	–	–	–	–	5.70 ± 0.09[b]
	M	CC	–	–	–	–	–
		CG	–	–	–	–	5.50 ± 0.10[a]
		GG	–	–	–	–	5.50 ± 0.08[a]

[a,b] Within a trait and sex, genotype means without a common superscript letter are statistically significantly different (P<0.05).
[c] Mean values were adjusted for farm. Estimate of the effect is expressed in units of the trait.

between the POMC SNPs, i.e. they were in, or very close to, complete linkage. Therefore, no extra information would be gained from fitting both SNPs in the model as both SNPs provide the same information. However, to confirm this we analysed the two POMC SNPs in a combined model. As the frequency of some genotype classes was too low to be included in the analysis three classes were compared (CC/CC, CT/CT and TT/TT). As predicted, whilst the effect of POMC on tenderometer score remained significant (P = 0.02), the size of the effect was the same as for the two SNPs analysed independently.

3.5.2. Haplotype analysis

Haplotypes with a frequency of ≤0.01 were excluded from the analysis; this left the following: CRH (4 haplotypes), GH (4), POU1F1 (2), POMC (3), PPARA (3), and PRKAG3 (4), details of which can be seen in Table 3. Using a nested haplotype model for each significant trait the F-statistics associated with haplotype group within SNP group were not significant. This indicates that haplotype information did not account for additional variation compared to a model with only genotype information.

4. Discussion

The specific aim of this study was to test, in a commercial population, whether there were significant associations between candidate genes identified in previous studies and meat and carcass quality traits. A comparison of previously seen associations and those found in the present study is given in Table 6. The results can be grouped into three categories as follows: 1). Previously reported effects (or closely related effects) confirmed, 2). Effects observed on alternative traits and 3). No effects observed for any trait.

Results for GH, MC4R and PPARA belong to the first of the three categories where previously seen associations (or those that are

closely related) were observed. GH, found on bovine chromosome 19 (Taylor et al., 1998), is a major regulator of post-natal growth and metabolism, and affects growth rate and body composition through the action of insulin-like growth factor 1 (IGF1) (Ge, Davis, Hines, Irvin, & Simmen, 2003). Therefore, it is a prime functional candidate through the predicted physiological effect on growth traits in livestock. Whilst polymorphisms in the gene have previously been shown to be associated with various carcass related traits, results are not always consistent. In Japanese Black cattle GH genotype has been found to be associated with both rib thickness (Ardiyanti et al., 2009) and hot carcass weight (Tatsuda et al., 2008). In the present study no association between GH genotype and hot carcass weight was found, however two of the polymorphisms tested were significantly associated with eye muscle length as a % of sirloin muscle length, eye muscle length and eye muscle area. Furthermore, an additional GH polymorphism approached significance for an effect on sirloin weight before maturation (P = 0.06). These results are in contrast to a study carried out in Continental × British hybrid beef steers by Sherman et al. (2008) where an alternative GH polymorphism was not significantly associated with any of the 19 carcass quality traits measured. Similarly, in Piedmontese cattle, polymorphisms in intron 3 and exon 5 of the GH gene did not have significant effects on various size- and weight-related traits (Di Stasio, Sartore, & Albera, 2002; Di Stasio et al., 2003).

A number of studies have reported an association between polymorphisms in GH and fat-related traits. Specifically, marbling in Australian feedlot and Japanese Black cattle (Barendse et al., 2006; Tatsuda et al., 2008), rump fat in Australian feedlot cattle (Barendse et al., 2006) and fat thickness in Landrace pigs (Franco et al., 2005). The effect of GH on these traits is not surprising as it has several functions over and above its effect on growth promotion, which include regulation of fat synthesis, stimulation of fatty acid

Table 6
Comparison of gene associations predicted from previously published studies and SNP-trait associations observed in the present study.

Gene	Predicted associations	Reference	Species	Observed associations (this study)	Genotype effect (SD units)
CRH	Subcutaneous fat depth	Wibowo et al. (2007)	Cattle		
	Beef marbling score		Cattle		
	End of test rib-eye area	Buchanan et al. (2005)	Cattle		
	Hot carcass weight		Cattle		
	Feed conversion ratio	Murani, Ponsuksili, Schellander, and Wimmers (2006)	Pigs		
	Carcass length		Pigs		
	Lean content		Pigs		
	Meat colour		Pigs		
				Gristle distance from fat band, mm	0.69
				pH at 24 h	0.64
GH	Rib thickness	Ardiyanti et al. (2009)	Cattle	Eye muscle length as a % of sirloin length	0.29
	Hot carcass weight	Tatsuda et al. (2008)	Cattle	Eye muscle area, mm^2	0.31
				Eye muscle length, mm	0.33
	Beef marbling	Tatsuda et al. (2008)	Cattle		
	Marbling	Barendse et al. (2006)	Cattle		
	Rump fat		Cattle		
	Fat thickness	Franco et al. (2005)	Pigs		
	Cook loss	Di Stasio et al. (2003)	Cattle		
	11 day tenderness		Cattle		
IGF2	Longissiumus muscle area	Sherman et al. (2008)	Cattle	None	
	Average daily gain		Cattle		
	Feed conversion ratio		Cattle		
	Fat %	Goodall & Schmutz, 2007	Cattle		
	Rib-eye area		Cattle		
	Muscle growth	Van Laere et al. (2003)	Pigs		
MC4R	Backfat depth	Houston et al. (2004)	Pigs	Fat class, transformed numerical scale	0.37
	Average daily gain		Pigs		
	Daily feed intake		Pigs		
PGAM2	Drip loss percent	Fontanesi et al. (2003)	Pigs	None	
POU1F1	Backfat	Yu et al. (1995)	Pigs		
	IMF	Thomas et al. (2007)	Cattle		
	Carcass length	Brunsch, Sternstein, Reinecke, and Bieniek (2002)	Pigs		
	Hot carcass weight		Pigs		
				Gristle length, mm	0.33
POMC	Shipping weight	Buchanan et al. (2005)	Cattle	Eye muscle length, mm	0.67
	Hot carcass weight		Cattle	Eye muscle area, mm^2	0.75
				Eye muscle length as a % of sirloin length	0.67
				Tenderometer score, kPa[5]	0.30
PPARA	Close to QTL for backfat	Szczerbal et al. (2007)	Pigs	pH at 24 h	0.31
				Sirloin weight after maturation, kg	0.33
				Sirloin fat depth, mm	0.50
				Juiciness	0.24
PRKAG3	Loin pH	Ciobanu et al. (2001)	Pigs		
	pH	Lindahl et al. (2004)	Pigs		
	Water holding capacity		Pigs		
	Cook loss		Pigs		
				Hot carcass weight, kg	0.33
				Gristle distance from eye muscle base, mm	0.60
SST	Marbling score	Morsci et al. (2006)	Cattle		
	Yearling height		Cattle		
				Eye muscle depth, mm	0.30

mobilisation from adipose tissues, fatty acid oxidation and regulation of protein turnover (Renaville, Hammadi, & Portetelle, 2002). However, surprisingly, the present study found no association between the *GH* polymorphisms and any of the fat-related traits tested. A significant effect has been previously observed between a *GH* intron 3 polymorphism and cooking loss and 11 day tenderness in Piedmontese cattle, where a gene substitution effect of 2.39% and 0.65 kg respectively was calculated (Di Stasio et al., 2003). In the present study, we found that a SNP in exon 5 approached significance for an effect on cooking loss (P = 0.06) and a SNP in intron 3 approached significance for an effect tenderometer score (P = 0.09) (data not shown). Further studies are needed to confirm or rule out a growth hormone effect on these quality traits.

The results for the *PPARA* gene bridge the gap between category one and category two, as previously published results were observed along with novel associations. For example, one of the SNPs, *A82G*, was associated with sirloin fat depth in the present study, a result that was perhaps expected due to its location close to a QTL for backfat in pigs (Szczerbal et al., 2007). A more novel association was observed

with the *C232G* SNP which was found to be associated with both pH at 24 h and juiciness as measured by the taste panel. As the majority of animals fell into only two genotype groups for this SNP (CG and GG) we were unable to separate the additive and dominance effects. In both cases animals with the GG genotype had higher trait values than animals with the CG genotype. The effect of this SNP on the taste panel assessed juiciness trait may be linked to water holding capacity (WHC) which is related to the degree of marbling (intramuscular fat) in the meat (Thompson, 2004). Additionally, it is known that WHC is influenced by muscle pH, where a fall in pH leads to a reduction in the power of muscle proteins to bind water. Meat with low WHC loses a lot of fluid during cooking and may be perceived by a consumer, or, in this case, a taste panel, as being dry and lacking in succulence (Warriss, 2000).

The results for *CRH*, *POU1F1*, *POMC*, *PRKAG3* and *SST* fall into the second category, i.e. completely novel gene–trait associations. Of these genes, the evidence for an effect on carcass quality traits is strongest for polymorphisms in the *CRH* gene. This gene is involved in appetite control (Marsh et al., 1999) and has been mapped to bovine

chromosome 14 (Barendse et al., 1997), close to a QTL for beef marbling score (Mizoshita et al., 2004) and subcutaneous fat depth (Casas et al., 2003, 2000). However, once again, previous association studies do not show consistent results. In Charolais-cross steers a polymorphism in the gene was linked to both hot carcass weight (P = 0.002) and end-of-test rib-eye area (P = 0.03) where the difference between the two homozygote genotype groups was 14.3 kg and 6 cm^2 respectively (Buchanan et al., 2005). Also in cattle, a number of *CRH* polymorphisms have been associated with both sirloin fat depth (P = 0.002) and beef marbling score (P = 0.02) (Wibowo et al., 2007), whilst in pigs variation in this gene has been associated with carcass length (P = 0.01) and lean content (P = 0.03) (Murani et al., 2006). In contrast to these results there were no significant associations with fat and carcass weight traits for the three SNPs tested in the present study. Similarly, Sherman et al. (2008) observed no association between polymorphisms in the gene and quality traits such as carcass weight, marbling score, backfat and yield grade, when tested in a group of experimental cattle. However, in the present study an association was observed between the *A145G* SNP and both gristle distance from fat band (in the sirloin steak) and pH at 24 h. Animals with the AA genotype had a pH that was 0.08 higher and gristle that was 4.33 mm further away from the sirloin fat than animals with the GG genotype. This SNP was also tested by both Buchanan et al. (2005) and Wibowo et al. (2007); however, measurements on pH level and gristle were not included in their analysis.

The *C254T* SNP in the *POMC* gene was found to be associated with eye muscle-related traits such as area and length. This SNP was previously found to be associated with both hot carcass weight and shipping weight but not rib-eye area (Buchanan et al., 2005). An additional novel association was observed with the two alternative *POMC* SNPs tested, which were in strong LD. In both cases animals with the TT genotype had a 2 kPa increase in tenderometer score, indicating an increase in meat toughness when compared to animals with the CC genotype. Furthermore, there was a significant association with taste panel assessed tenderness for both SNPs, however, this association was not significantly additive and the CT animals were found to be the most tender with the CC and TT animals scoring equally. This discrepancy could be due to the subtle differences between tenderometer and taste panel scoring. The human interpretation of tenderness is not simply related to the force required to shear meat (as in the tenderometer machine), but includes factors such as the rate at which fibres are broken down. Therefore, a simple physical measurement of shear force cannot incorporate all of the features of a human-based evaluation (Warriss, 2000).

There were two genes where associations with traits have been reported in previous studies but showed no associations with the traits reported in the present study: *IGF2* and *PGAM2*. Of these, the previous evidence for an association with carcass quality traits is more convincing for the *IGF2* gene. This gene produces a 67-amino acid peptide hormone reported to have effects on post-natal growth, metabolism and body weight regulation (DeChiara, Efstratiadis, & Robertson, 1990). Additionally, the gene maps to bovine chromosome 29 (Schmutz, Moker, Gallagher, Kappes, & Womack, 1996), close to QTL for hot carcass weight (Casas, Keele, Shackelford, Koohmaraie, & Stone, 2004; MacNeil & Grosz, 2002) and marbling (MacNeil & Grosz, 2002) and a regulatory mutation in the gene has been shown to cause a major QTL effect on muscle growth in pigs (Van Laere et al., 2003). Furthermore, the *C150T* SNP in exon two of the gene has been significantly associated with rib-eye area, such that animals with the TT genotype had rib-eye areas 22.3 cm^2 smaller than animals with the CC genotype (Goodall & Schmutz, 2007). In contrast, a more recent study found that animals with the TT genotype at the same SNP had 6.53 cm^2 larger rib-eye areas (longissimus muscle area) than animals with the CC genotype (Sherman et al., 2008). The authors suggest that this inconsistency may be due to a lack of animals with the TT

genotype in the small sample number tested (n = 11). In the present study there were only 24 animals with the TT genotype giving a minor allele frequency of 0.19, and it is possible that the study was underpowered for detecting small effects.

The lack of consistency between studies may be due to a number of factors, e.g. differences in the population under examination and genetic background may influence the size or direction of the effects of a particular gene variation. Furthermore, differences in the way animals are managed, the way traits are measured across studies, different marker-causative mutation linkage phases, genotype-by-environment interactions and sample size could all influence the specific study results.

Haplotype analysis was performed to assess whether incorporating information on combinations of SNPs (for *CRH*, *GH*, *MC4R*, *POU1F1*, *POMC*, *PPARA* and *PRKAG3*) improved the models compared with single SNP genotypes. Using haplotypes in the trait analysis accounted for little extra variation for any of the SNP/trait combinations. This may be because either (i) only the significant SNP was in LD with the causative mutation, or (ii) the SNPs were themselves in strong LD. Consequently, constructing haplotypes did not define chromosome segments in stronger LD with the causative mutation than the most significant SNP. As a result incorporating haplotype information for these genes would not improve the performance of marker-assisted selection for this population.

5. Conclusions

In the present study some of the previously reported associations between genes and meat production related traits were confirmed. In addition a number of novel associations between candidate genes and economically important carcass and meat quality traits were found. However some of the SNP-trait associations previously reported were not seen. This lack of consistency between studies confirms the importance of continued validation of SNP effects in different populations and breeds before implementation in breeding programs. In addition, it is important that we fully understand the mechanism of action of these polymorphisms on the traits in question, particularly those that do not cause amino acid changes but may be linked to other, as yet unknown, causative mutations.

When the effects of polymorphisms on particular traits are validated, they may be used in marker-assisted selection programs to increase the rate of trait improvement in difficult and expensive to measure traits such as aspects of meat quality, particularly in cases where genome-wide predictions are not available.

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