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Quantitative trait loci with additive effects on palatability and fatty acid composition of meat in a Wagyu–Limousin F_2 population

L. J. Alexander*, M. D. MacNeil*, T. W. Geary*, W. M. Snelling†, D. C. Rule‡ and J. A. Scanga§

*USDA-ARS, Miles City, MT 59301, USA. †USDA-ARS, Clay Center, NE 68933, USA. ‡University of Wyoming, Laramie, WY 82071, USA. §Colorado State University, Fort Collins, CO 80523, USA

Summary

A whole-genome scan was conducted on 328 F_2 progeny in a Wagyu × Limousin cross to identify quantitative trait loci (QTL) affecting palatability and fatty acid composition of beef at an age-constant endpoint. We have identified seven QTL on five chromosomes involved in lipid metabolism and tenderness. None of the genes encoding major enzymes involved in fatty acid metabolism, such as *fatty acid synthase* (*FASN*), *acetyl-CoA carboxylase alpha* (*ACACA*), *solute carrier family 2 (facilitated glucose transporter) member 4* (*SLC2A4*), *stearoyl-CoA desaturase* (*SCD*) and genes encoding the subunits of fatty acid elongase, was located in these QTL regions. The present study may lead to a better-tasting and healthier product for consumers through improved selection for palatability and lipid content of beef.

Keywords cattle, fatty acid, Limousin, lipid, quantitative trait loci, Wagyu.

Introduction

Ideally, consumers of beef want a palatable and healthy product at a reasonable price. As consumers become more health conscious, they are more concerned about the levels of saturated fat (SFA) in their diet. Relative to other breeds, Wagyu beef has more marbling and an increased ratio of monounsaturated (MUFA) fat to SFA (May *et al.* 1993; Elias Calles *et al.* 2000). This increased ratio has human health implications in terms of overall health and decreasing coronary heart disease (McDonald 1991). Also, conjugated linoleic acid (CLA) has been shown to decrease fat and total body weight, total and LDL cholesterol in animal models while in humans there are conflicting data on the positive effects of CLA (for a review, see Salas-Salvado *et al.* 2006). In addition, composition of beef fat also has implications in product taste with oleate being positively correlated with fat softness (Smith *et al.* 1998) and flavour scores (Dryden & Marchello 1970; Westerling & Hendrick 1979; Melton *et al.* 1982).

In a previous study, Wagyu-sired crossbred steers had 19% greater fat depth at the P8 site, and meat from the longissimus sampled at the 10th–11th rib had 45% greater intramuscular fat (IMF) content and 4% greater MUFA content than Limousin-sired cohorts at marketable weights (Pitchford *et al.* 2002). Further, Mir *et al.* (2002) found that Wagyu steers had 9% more subcutaneous fat over the 12th rib, and meat from the longissimus sampled at that point had 116% more IMF and 5% greater MUFA content than Limousin steers at a *quasi* weight constant endpoint. These differences between the breeds suggest Limousin and Wagyu as candidate breeds from which to develop populations for mapping quantitative trait loci (QTL) that affect healthfulness and palatability of beef.

Many previously identified bovine QTL have been summarized in http://bovineqtlv2.tamu.edu/index.html and http://www.animalgenome.org/QTLdb. Additionally, Mizoshita *et al.* (2004) and Mizoguchi *et al.* (2006) performed genome-wide scans for QTL effecting carcass traits in purebred Wagyu populations.

In this study, we performed a whole-genome scan on 328 F_2 progeny in a Wagyu × Limousin cross derived from eight founder bulls. We identified seven QTL on five chromosomes involved in lipid metabolism and tenderness. Application of the results presented here may lead to a better-tasting and healthier product for consumers.

Materials and methods

Animal management

Wagyu–Limousin F_1 bulls and females were purchased from Washington State University in October, 1999. Eight Wagyu bulls were mated with 108 Limousin females to

Address for correspondence

L. J. Alexander, USDA-ARS, LARRL, Ft Keogh, 243 Fort Keogh Road, Miles City, MT 59301, USA.
E-mail: lee.alexander@ars.usda.gov

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produce 121 F_1 females over a 3-year period, and three of the original eight bulls sired the six F_1 bulls used. In this project, the F_1 animals were *inter se* mated, except that mating of known relatives was avoided, to produce 328 F_2 progeny that were born in 2000–2003.

Calves were reared by their dams, without creep feed, until weaning at approximately 175 days of age (SD: 14 days). Each year, before harvest, calves were randomly assigned separately by sex to a harvest date in groups of 8–11 head per day. After weaning, the calves were managed in a two-phase system: a growing phase with diet composition of 50–54% DM, 14.4–15.6% CP and 1.06–1.18 Mcal/kg net energy of gain (NEg), and a finishing phase with diet composition 68–70% DM, 11.6–13.4% CP and 1.26–1.31 Mcal/kg NEg. The finishing diet was fed a minimum of 113 days until the calves were harvested. Groups of calves were harvested at 2- to 3-week intervals. Thus, the final group harvested each year had been fed the finishing diet at least 210 days.

Calves, 450–641 days of age (average 561 days), were transported to the abattoir the afternoon before harvest, held overnight with water and without feed, and harvested the next morning using standard industry procedures. Hot carcass weight was measured immediately following harvest and prior to carcass rinsing/washing and chilling. Marbling score, fat thickness and percent kidney-pelvic-heart fat (KPH) were assessed by a trained evaluator after 48 h of chilling at 2 °C. Fat thickness was recorded at the 12th rib at a point three-fourths the width of the longissimus muscle from its chine bone end. Marbling (4.00 = slight^0, 5.00 = small^0, 6.00 = modest^0, 7.00 = moderate^0, etc.) was determined at the interface of the 12th and 13th ribs and was evaluated by subjective comparison of the amount of fat within the longissimus muscle with photographic standards (National Livestock and Meat Board 1981). The amount of KPH was estimated and recorded as a percentage of carcass weight.

After the carcasses were evaluated, wholesale 107-rib cut (North American Meat Processors Association 2002) was removed from each carcass, vacuum-packaged and aged for 14 days at 2 °C. After ageing, a three-rib section and four 2.54-cm-thick steaks were cut from the posterior end of the wholesale rib, then individually vacuum-packaged, frozen at −20 °C and held for further analyses.

The frozen rib sections were transported to Colorado State University (CSU) for use in sensory and shear force evaluation. Upon receipt at CSU, products were kept frozen (−20 °C) prior to evaluation. Frozen rib sections (ribs 9–11) were removed from their original packaging and three 2.54-cm-thick steaks were cut from the distal end. Frozen steaks were vacuum-packaged and returned to frozen storage (−20 °C). In preparation for tenderness evaluation, frozen steaks were removed from the freezer and tempered (2 °C) for 24–26 h prior to cooking.

Warner–Bratzler shear force determination

Steaks were cooked on an electric conveyor grill (model TGB-60; Magikitch'n) for 6 min and 35 s at a setting of 176 °C to a target internal temperature of 70 °C. After cooking, each steak was allowed to equilibrate to room temperature (22 °C) and 6–10 cores (1.27 cm in diameter) were removed from each steak parallel to the muscle fiber orientation. Each core was sheared once, perpendicular to the muscle fiber orientation, with an Instron testing machine fitted with a Warner–Bratzler shear head. Peak shear force measurements of each core were recorded and averaged to obtain a single shear force value (Warner–Bratzler shear force; WBSF) for each steak.

Trained sensory panel

Steaks were cooked as previously described and portioned into uniform pieces (1.3 × 1.3 × 1.9 cm) for sensory panel evaluation. Sensory samples were served warm (approximately 50 °C) to an eight-member panel. Panelists were selected and trained according to procedures outlined by American Meat Science Association (1995). Panelists scored each sample on an 8-point scale for initial juiciness, muscle fibre tenderness, connective tissue amount, overall tenderness and beef flavour intensity (8 = extremely juicy, extremely tender, none, extremely tender, extremely beefy for these characteristics respectively; 1 = extremely dry, extremely tough, abundant, extremely tough, extremely bland for these characteristics respectively).

Fatty acid analysis

Two steaks from each carcass were transported to University of Wyoming for the determination of fatty acid composition as described by Rule *et al.* (2002). Briefly, the entire core of the longissimus dorsi was sampled (i.e. devoid of trim fat and extraneous muscles) by dicing the muscle into 1.0-cm cubes while the muscle was semi-frozen and then weighed into pre-weighed, plastic cups with perforated lids. All samples were freeze-dried (Genesis 25 freeze dryer; The VirTis Co.) and then ground and homogenized using a home-style electric coffee grinder. Samples were packed into 20-ml plastic vials and sealed to inhibit exposure to air, and then stored at −80 °C until analysed for fatty acids and cholesterol, which occurred within 2 to 4 weeks of freeze-drying. Approximately 150 mg of dried muscle was weighed, in duplicate, into 16 × 125-mm screw-capped tubes that contained 1.0 mg of tridecanoic acid as internal standard, and then subjected to direct saponification as described by Rule *et al.* (2002). Samples were reacted with 4.0 ml of 1.18 M KOH in ethanol at 90 °C along with frequent vortex-mixing (two to three times per minute for 3 s each time) until the sample was completely dissolved, except for insoluble collagen that appeared as a white

powder in suspension upon mixing. After about 45 min, tubes were cooled, 2.0 ml of water was added and cholesterol was extracted with 2.0 ml of hexane that contained 0.1 mg/ml of stigmasterol as internal standard for the cholesterol assay; the hexane phase was transferred to gas liquid chromatography (GLC) vials and sealed. One millilitre of concentrated HCl was added to the original tubes and fatty acids were extracted in 2.0 ml of hexane for fatty acid methyl ester (FAME) preparation, which was carried out according to Rule *et al.* (2002) using methanolic HCl as a catalyst. Analysis of CLA is hampered by use of acid catalysts because of partial geometric isomerization of *cis*-9, *trans*-11 CLA to *trans*-9, *trans*-11 CLA (Yamasake *et al.* 1999) and degradation of CLA to allylic methoxy artifacts (Kramer *et al.* 1997). However, Murrieta *et al.* (2003) demonstrated that dietary treatment effects on CLA in ovine muscle were maintained when acid catalysts were used for FAME preparation, despite up to 20% loss of *cis*-9, *trans*-11 CLA. Preparation of FAME from nonesterified free fatty acids (NEFA) requires the use of the acid catalyst because alkaline catalysts do not react with NEFA to form FAMEs (Christie 1982). For the current study, freeze-dried muscle samples were chosen at random from approximately 5% of the samples for FAME preparation using methanolic KOH, which does not affect CLA proportions. We did not observe losses of CLA in the samples analysed (data not shown). Generally, we have observed either minimal or no loss of CLA in samples containing low concentrations (about 0.5 mg per 100 mg of total fatty acids) of this fatty acid. Cholesterol concentration was determined using GLC as described by Rule *et al.* (1997), and fatty acids were analysed by GLC as described by Murrieta *et al.* (2003).

Genotyping

DNA was extracted from semen of sires of the F_1 bulls and from white blood cells of the F_1 bulls, F_1 females and F_2 calves using standard protocols (e.g. Ausubel *et al.* 1994). Touchdown PCR was performed in MJ Research thermocyclers as described previously (MacNeil & Grosz 2002). All genotypes were collected on a LiCor 4200 DNA Analysis System. Genotypes were independently scored by two individuals. PCR was repeated for discrepancies that could not be resolved. Anomalous genotypes were detected using GENOPROB (Thallman *et al.* 2001a,b). Genetic linkage maps were constructed using CRI-MAP (Green *et al.* 1990; http://compgen.rutgers.edu/multimap/crimap/). Initially, 156 markers covering the 29 bovine autosomes were chosen from http://www.marc.usda.gov/genome/cattle/cattle.html based on marker position, suitability for multiplex reactions, ease of scoring and number of alleles. Fine-mapping was performed by adding an additional 61 markers in areas of the genome exhibiting the presence of a QTL.

Data analyses

Quantities of the various fatty acids were summarized by calculating total amounts per 100 mg dry tissue of SFA MUFA, polyunsaturated fatty acids (PUFA) and three indexes of Δ^9 desaturase activity (R_1 = 14:1 to 14:0; R_2 = 16:1 to 16:0; R_3 = 18:1 to 18:0). Amounts of CLA and cholesterol were analysed separately.

QTL were identified by least squares regression analysis using the F2 analysis option of the QTL Express program (Seaton *et al.* 2002; http://qtl.cap.ed.ac.uk/) which generated an *F*-statistic profile at 1-cM intervals for each chromosome. For each chromosome, the effect of a single additive QTL was modelled with simultaneous adjustment for classification effects of year and sex, and the continuous linear effect of age at harvest. Given the random assignment of animals to harvest dates and the statistical model, the data were adjusted to an age-constant endpoint. For newly identified QTL, the observed significance level was adjusted to a genome-wide basis following the procedure described by Cheverud (2001). The observed significance level was used to confirm previously identified QTL.

To identify positional candidate genes underlying QTL, bacterial artificial chromosome (BAC) contigs corresponding to QTL were identified from the bovine composite map (W. M. Snelling *et al.* in preparation; http://genomes.tamu.edu/cgi-bin/gbrowse/bosmap2/). Syntenic regions defined by the bovine BAC-human comparative map were determined, and a list of Ensembl genes was extracted using BioMart (http://www.ensembl.org/Homo_sapiens/index.html) and summarized with the Gene Ontology Tree Machine (Zhang *et al.* 2004; http://bioinfo.vanderbilt.edu/gotm/).

Results and discussion

Initially, 157 microsatellite markers that covered the 29 autosomes were chosen from the USMARC genetic linkage map (http://www.marc.usda.gov/genome/cattle/cattle.html) (Table S1). An additional 61 markers were added in regions that exhibited potential QTL. Most of the maps generated in this study were in good agreement with the USMARC bovine linkage maps, with the exception that some chromosomes were inflated in length. Chromosomes whose length were more than 15% greater than indicated by the USMARC map were BTA2 (26%), 9 (16%), 12 (19%), 13 (26%), 14 (22%), 18 (23%) 20 (21%) and 27 (20%) (Table S1).

Mean values and standard deviations for the phenotypes evaluated in this study are shown in Table 1. Six new QTL involved in lipid metabolism and deposition were identified on four chromosomes during this study and are summarized in Table 2.

A QTL with multi-faceted significant effects on CLA [F = 12.01, P = 0.013 (genome-wide significance)], marbling

Table 1 Means and standard deviations of phenotypes examined in this study.

Phenotype	$\bar{X}$	SD
Cholesterol (mg/100 g dry tissue)	218	21
CLA (mg/100 g dry tissue)	43.3	15.8
Subcutaneous fat depth (mm)	0.39	0.16
KPH (%)	2.65	0.35
Marbling	6.07	1.2
MUFA (mg/100 g dry tissue)	10 484	3077
SFA (mg/100 g dry tissue)	9033	2607
PUFA (mg/100 g dry tissue)	883	130
R_1 – ratio of C14:1 to C14:0	0.276	0.073
R_2 – ratio of C16:1 to C16:0	0.146	0.018
R_3 – ratio of C18:1 to C18:0	3.73	0.47
Warner–Bratzler shear force (WBSF), kg	3.94	0.69
Connective tissue	7.26	0.36
Myo-fibral tenderness	5.89	0.73
Overall tenderness	5.88	0.72
Flavour	5.61	0.37
Juiciness	5.93	0.46

CLA, conjugated linoleic acid; KPH, kidney–pelvic–heart fat; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid.

($F = 11.76$, $P = 0.015$), MUFA ($F = 21.37$, $P = 0.00012$), SFA ($F = 12.52$, $P = 0.01$), R_2 ($F = 19.44$, $P = 0.00032$), R_3 ($F = 25.18$, $P = 0.00002$) and a suggestive effect on flavour ($F = 7.91$, $P = 0.11$) were observed towards the centromere of BTA2 (Fig. 1). Information content of individual markers in this region ranged from 0.24 to 0.63. These QTL effects are seemingly consistent with a unified biological model supportive of a gene or genes segregating between Wagyu and Limousin in this region that confer greater lipid content, greater stearoyl-CoA desaturase-like activity and a more desirably flavoured steak on the Wagyu breed. Flavour has been positively correlated with the MUFA oleate (Dryden & Marchello 1970; Westerling & Hendrick 1979; Melton *et al.* 1982) and the subjectivity and categorical scale of its measurement may compromise the ability to detect QTL effects. Casas *et al.* (1998) and MacNeil & Grosz (2002) identified QTL with effects on KPH fat in this region. Also, Casas *et al.* (1998) previously reported a co-located QTL for marbling score. Taken together, these results strongly suggest the centromeric region of BTA2 as a candidate for further investigation. The QTL region corresponds to contig ctg2005 on the international bovine BAC map (http://www.bcgsc.ca/downloads/bovinemap.tar.gz), which is syntenic with the 168-to-191-Mb region of HSA2, which contains nearly 200 genes.

A QTL with an effect on MUFA (125 cM, $F = 10.76$, $P = 0.025$) was observed on BTA7 (Fig. 2). Information content of individual markers in this region ranged from 0.02 to 0.60. This QTL region also affected R_2, R_3, SFA and CLA, although these traits only had genome-wide significance levels of $P = 0.057$, 0.076, 0.065 and 0.092 respectively. A second suggestive QTL affecting KPH (40 cM, $F = 8.16$, $P = 0.096$) was observed at 40 cM on our BTA7 map, and covers portions of the bovine genome syntenic with HSA5 and HSA19. Information content of individual markers in this region ranged from 0.29 to 0.56. The 2-to-19-Mb region of HSA19, corresponding to the KPH fat QTL, contains 21 genes with lipid-related function. No lipid-related genes are in the 128-to-131-Mb region of HSA5 under the KPH QTL, although the 89-to-110-Mb region of HSA5 corresponding to the R_2, R_3 and MUFA QTL contains three genes related to lipid and phospholipid binding: *RGM domain family member B* (*RGMB*), *multiple C2 domains transmembrane protein* (*MCTP1*) and *ephrin-A5* (*EFNA5*). To our knowledge, neither of these QTL has been reported before. However, Casas *et al.* (2003) reported a QTL with effects on fat thickness in the interval between 44 and 71 cM on BTA7. While not as compelling as the region on BTA2, these results suggest that BTA7 merits further investigation for genes affecting lipid deposition and metabolism. BTA12 also had a QTL region near the centromere that slightly affected SFA and MUFA (BTA12, $F = 9.43$, $P = 0.052$ and $F = 7.66$, $P = 0.128$ respectively) and R_2 (BTA28, $F = 9.29$, $P = 0.11$). The substitution of a Wagyu allele replacing a Limousin allele (Table 2) promoted more MUFA and SFA on BTA2 where on BTA7 and 12 the Wagyu alleles repressed MUFA and SFA.

The centromeric region of BTA5 had a QTL influencing myofibril ($F = 12.66$, $P = 0.008$) and overall tenderness ($F = 10.17$, $P = 0.030$). Information content of individual markers in this region ranged from 0.38 to 0.83. Casas *et al.* (2000) also found a QTL for tenderness on BTA5; however, their location was at 65 cM. Other suggestive QTL affecting subcutaneous fat thickness (BTA1, $F = 9.7$, $P = 0.051$) and WBSF (BTA10, $F = 7.90$, $P = 0.117$) were observed in this study. Casas *et al.* (2003) also found a QTL affecting fat thickness in the same area of BTA1 as reported here. Because of the reputation of the palatability of Wagyu meat, it was somewhat surprising that no significant differences were detected by the sensory panel for flavour and juiciness. This lack of difference has been reported before between Angus and crossbred (3/4 and 7/8) Wagyu steers (May *et al.* 1993). However, Elias Calles *et al.* (2000) found in F_1 crosses a positive correlation between marbling and flavour, tenderness and juiciness using 'Old' and 'New' Wagyu bulls mated with either Angus or Angus × Hereford × Simmental crosses. Other phenotypes for which QTL were not detected are also listed in Table 2.

Mizoshita *et al.* (2004) and Mizoguchi *et al.* (2006) previously reported genome scans using purebred Wagyu germplasm and found QTL for marbling (BTA4, 6 and 21) and subcutaneous fat depth (BTA13). Data from the present study confirmed the above centrally

Table 2 Location of QTL affecting lipid deposition, profile and meat characteristic traits in a Wagyu × Limousin cattle population.

Trait[1]	BTA[2]	Position cM	95% CI[3]	Start CI	End CI	*F*-statistic	Significance[4]		Additive effect ± S.E[5]	Flanking marker, position (cM)
							Nominal	Genome-wide		
Fat_d	1	58	46	35	81	9.7	0.002	0.051	−0.053 ± 0.017	*BMS4030*, 48.7; *DIK2121*, 62.4
CLA	2	2	46	0	25	12.01	0.001	0.013	5.265 ± 1.519	*TGLA44*, 0.00; *DIK4469*, 3.50; *ILSTS026*, 9.7
Flavour	2	0	70	0	35	7.91	0.005	0.11*	0.096 ± 0.035	*TGLA44*, 0.00; *DIK4469*, 3.50
KPH	2	25	55	0	53	9.33	0.002	0.054	0.105 ± 0.034	*DIK1172*, 21.7; *CSFM050*, 24.90; *TEXAN-2*, 35.40
Marbling	2	3	49	0	28	11.76	0.001	0.015	0.400 ± 0.117	*DIK4469*, 3.50; *ILSTS026*, 9.7
MUFA	2	5	24	0	17	21.37	5.66E-06	1.26E-04	1374.7 ± 297.3	*TGLA44*, 0.00; *DIK4469*, 3.50; *ILSTS026*, 9.7
SFA	2	10	40	0	30	12.52	0.000	0.010	919.8 ± 260.0	*DIK4469*, 3.50; *ILSTS026*, 9.7
R_2	2	3	28	0	17	19.44	1.45E-05	3.24E-04	0.778 ± 0.176	*DIK4469*, 3.50; *ILSTS026*, 9.70; *TGLA431*, 12.7
R_3	2	4	21	0	15	25.18	9.03E-07	2.01E-05	22.445 ± 4.473	*TGLA44*, 0.00; *DIK4469*, 3.50; *ILSTS026*, 9.7
Myo-fibral tenderness	5	0	44	0	22	12.66	0.0004	0.008	−0.246 ± 0.069	*BMS1095*, 0; *BM6026*, 10.4
Overall tenderness	5	0	58	0	29	10.17	0.002	0.030	−0.216 ± 0.068	*BMS1095*, 0; *BM6026*, 10.4
CLA	7	131	47	108	end	8.24	0.004	0.092	−5.195 ± 1.810	*CST*, 122; *BMS1979*, 133.4
KPH	7	40	77	2	79	8.16	0.005	0.096	−0.090 ± 0.031	*BMS713*, 21.1; *BM6105*, 40.3; *TGLA303*, 42.2
MUFA	7	125	48	101	end	10.76	0.001	0.025	−1095.8 ± 334.0	*CST*, 122; *BMS1979*, 133.4
SFA	7	125	40	105	end	8.93	0.003	0.065	−848.5 ± 283.9	*CST*, 122; *BMS1979*, 133.4
R_2	7	110	55	83	end	9.17	0.003	0.057	−0.547 ± 0.181	*BM9065*, 99.7; *DIK4838*, 110.5; *CST*, 122
R_3	7	108	62	77	end	8.62	0.004	0.076	−13.4 ± 4.58	*BM9065*, 99.7; *DIK4838*, 110.5; *CST*, 122
CLA	7	131	47	108	end	8.24	0.004	0.092	−5.195 ± 1.810	*CST*, 122; *BMS1979*, 133.4
WBSF	10	95	21	85	end	7.9	0.005	0.117*	−0.316 ± 0.112	*BMS2641*, 79.2; *BL1134*, 110.9
MUFA	12	0	66	0	33	7.66	0.006	0.128*	−766.7 ± 277.0	*BMS410*, 0; *DIK2916*, 7.90
SFA	12	0	82	0	41	9.43	0.002	0.052	−718.7 ± 234.0	*BMS410*, 0.00; *DIK2916*, 7.90
R_2	28	50	11	45	end	9.29	0.003	0.11*	1.247 ± 0.409	*BM7246*, 33.4; *MB023*, 50.5 (end)
Cholesterol	ND									
Connective tissue	ND									
Juiciness	ND									
PUFA	ND									
R_1	ND									

[1]Fat_d, subcutaneous fat depth; CLA, conjugated linoleic acid; KPH, kidney–pelvic–heart fat; MUFA, amount of monounsaturated fat; R_1, ratio of C14:1 to C14:0; R_2, ratio of C16:1 to C16:0; R_3, ratio of C18:1 to C18:0; SFA, amount of saturated fat; WBSF, Warner–Bratzler shear force.

[2]ND, no suggestive or significant association detected.

[3]Confidence interval, calculated by the method of Darvasi & Soller (1997).

[4]Significant <0.05, suggestive <0.1; *approaching suggestive value.

[5]Effect resulting from a Wagyu allele replacing an allele from Limousin.

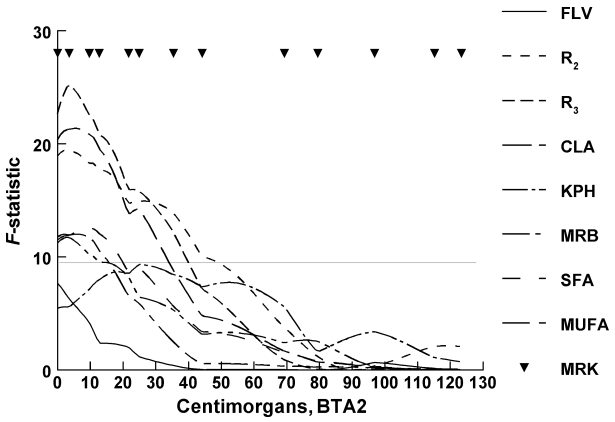

Figure 1 BTA2 *F*-statistic profiles for flavour (FLV), ratio of C16:1 to C16:0 fatty acids (R2), ratio of C18:1 to C18:0 fatty acids (R3), conjugated linoleic acid (CLA), per cent kidney–pelvic–heart fat (KPH), marbling score (MRB), saturated fatty acids (SFA), and monounsaturated fatty acids (MUFA). From centromere to telomere, markers were *TGLA44, DIK4469, ILSTS026, TGLA431, DIK1172, CSFM050, TEXAN-2, TGLA377, RM356, ILSTS050, BMS1866, BMS2267* and *BM2113* (shown as triangles). The genome-wide significance threshold of 9.46 is shown as a horizontal line.

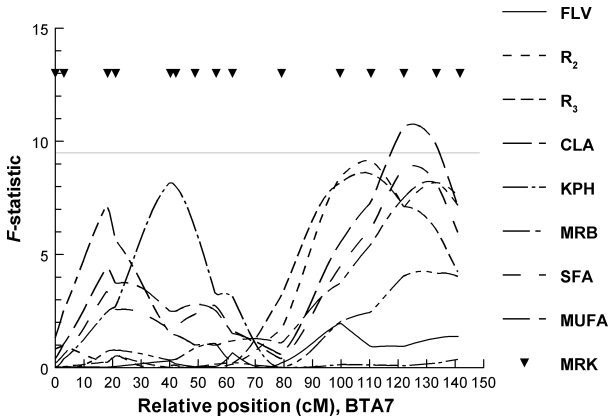

Figure 2 BTA7 *F*-statistic profiles for flavour (FLV), ratio of C16:1 to C16:0 fatty acids (R2), ratio of C18:1 to C18:0 fatty acids (R3), conjugated linoleic acid (CLA), per cent kidney–pelvic–heart fat (KPH), marbling score (MRB), saturated fatty acids (SFA), and monounsaturated fatty acids (MUFA). From centromere to telomere, markers were *BM7160, DIK2870, BL1067, BMS713, BM6105, TGLA303, DIK2819, TGLA164, UWCA20, BMS2258, BM9065, DIK4838, CST, BMS1979* and *BMS1247* (shown as triangles). The genome-wide significance threshold of 9.40 is shown as a horizontal line.

located QTL for marbling on BTA4 (max $F = 5.32$ at 66 cM, $P_{\text{nominal significance (ns)}} = 0.02$). However, these data do not confirm the other QTL for marbling and subcutaneous fat depth reported by Mizoshita *et al.* (2004) and Mizoguchi *et al.* (2006). The lack of correspondence of QTL between the previously reported projects and the present study may result from numerous differences between our experiments. Mizoshita *et al.* (2004) and Mizoguchi *et al.* (2006) searched for QTL that segregated within Wagyu, whereas we searched for QTL that segregated between Wagyu and Limousin. Substantive differences also exist between the studies in feeding strategies; in the present study, cattle were harvested at younger ages (15–21 months) than in the studies of Mizoshita *et al.* (2004); ~30 months) and Mizoguchi *et al.* (2006; 29–32 months).

To evaluate genes encoding enzymes involved in fatty acid metabolism as candidates for QTL observed here, we examined their genomic locations. *Fatty acid synthase* (*FASN*; Roy *et al.* 2001) and *acetyl-CoA carboxylase alpha* (*ACACA*; Mao *et al.* 2001), which are involved in *de novo* synthesis of fatty acids, were located on BTA19 while *acetyl-CoA carboxylase beta* (*ACACB*) was placed on BTA16 and BTA17 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj&cmd=Retrieve&dopt=Overview&list_uids=10708). *Solute carrier family 2 (facilitated glucose transporter), member 4* (*SLC2A4*), previously known as *GLUT4*, facilitates uptake of glucose that may be used in *de novo* fatty acid synthesis by muscle and adipose tissues and was also present on BTA19 (http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9913&query=GLUT4&qchr=&advsrch=off).

An obvious candidate gene for several of the observed differences in MUFA:SFA is bovine *stearoyl-CoA desaturase* (*SCD*). Campbell *et al.* (2001) mapped *SCD* (AF188710) to BTA26q21. A cDNA sequence has been isolated for bovine *stearoyl-CoA desaturase* 5 (*SCD5*) (BC112711). Its genomic location was not certain as conflicting positions for this gene are on BTA6 and BTA11.

Thus, the locations of *FASN, ACACA, ACACB, SLC2A4, SCD* and *SCD5* exclude them as candidates for the observations reported here. Another candidate enzyme may be fatty acid elongase. Subunits of this enzyme were identified using the Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/). None of the genes encoding the subunits was near the QTL reported here; however, one subunit, *hydroxysteroid (17-beta) dehydrogenase 10* (*HSD17B10*), previously known as *HADH2*, has not been placed in the bovine genome sequence.

Previous genome scans using purebred Wagyu found QTL for marbling and subcutaneous fat depth on BTA4, 6 and 21 (Mizoshita *et al.* 2004; Mizoguchi *et al.* 2006). In addition, some candidate genes for marbling and subcutaneous fat depth, *leptin* (*LEP*) on BTA4; *thyroglobulin* (*TG*), *diacylglycerol O-acyltransferase* (*DGAT1*, Winter *et al.* 2002; De *et al.* 2004) and *fatty acid binding protein* (*FABP4*, Michal *et al.* 2006) on BTA14; *growth hormone* (*GH*) on BTA19 and *mitochondrial transcription factor A* (*TFAM*, Jiang *et al.* 2005) on BTA28 have been explored in this Wagyu × Limousin population. These present data cannot exclude the effects of *LEP* on BTA4 ($F = 5.32$, $P_{\text{ns}} = 0.02$), *FABP4* on BTA14 ($F = 3.98$, $P_{\text{ns}} = 0.05$) or *GH* on BTA19 ($F = 3.29$, $P_{\text{ns}} = 0.07$) on marbling. The *GH* locus (BTA19) has also been reported to affect fatty acid composition of beef (Taylor *et al.* 1998).

In summary, we have identified seven new QTL regions on five chromosomes that influence tenderness, fatty acid deposition and profiles and meat quality in an F_2 Wagyu × Limousin population. Further investigation of these QTL will promote our understanding of lipid metabolism and fat deposition in cattle. As the consumer becomes more health conscious they have increased concerns about the levels of SFA in their diet. Therefore, CLA and the ratio of MUFA to SFA have human health implications in terms of overall health and coronary heart disease.

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Supplementary Material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2007.01643.x

Table S1 Mapping positions of the markers used in this study.

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