

Fatty acid synthase effects on bovine adipose fat and milk fat

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Abstract

A quantitative trait locus (QTL) was identified by linkage analysis on bovine Chromosome 19 that affects the fatty acid, myristic acid (C14:0), in subcutaneous adipose tissue of pasture-fed beef cattle (99% level: experiment-wise significance). The QTL was also shown to have significant effects on ten fatty acids in the milk fat of pasture-fed dairy cattle. A positional candidate gene for this QTL was identified as fatty acid synthase (*FASN*), which is a multifunctional enzyme with a central role in the metabolism of lipids. Five single nucleotide polymorphisms (SNPs) were identified in the bovine *FASN* gene, and animals were genotyped for *FASN* SNPs in three different cattle resource populations. Linkage and association mapping results using these SNPs were consistent with *FASN* being the gene underlying the QTL. SNP substitution effects for C14:0 percentage were found to have an effect in the opposite direction in adipose fat to that in milk fat. It is concluded that SNPs in the bovine *FASN* gene are associated with variation in the fatty acid composition of adipose fat and milk fat.

Introduction

Dietary fatty acids are known to have a major influence on human health, particularly in countries

whose populations are well-nourished, such as in developed countries where milk and meat are major components of dietary intake. It is generally accepted that saturated fats containing fatty acids with a chain length of 14 or 16 carbons, commonly found in beef meat and cow's milk, are risk factors in coronary heart disease (Mozaffarian et al. 2005). For example, myristic acid (C14:0) is hypercholesterolemic and raises concentrations of both low-density lipoprotein and high-density lipoprotein cholesterol compared with oleic acid (C18:1) (Temme et al. 1997). In contrast, those fatty acids with a carbon chain length of at least 18, especially unsaturated fatty acids, are considered to be beneficial to human health.

Methods are available to modify fatty acid composition in animal products that contain oil or fat, including chemical extraction, blending, and also specific feeding regimens for cattle (Baumgard et al. 2005). However, such methods are likely to incur a significant extra cost in producing these animal fats, which are relatively low-value commodity products. The methods may also compromise flavor, mouth feel, or other physical properties of the animal products (Berner 1993). In contrast to the farm livestock industry, the plant industry has been very successful in manipulating or tailoring fatty acid composition, especially in seed oil, via breeding or transgenics to improve edible traits and oils for industrial use (Davies 1996; Singh et al. 2005). Is a genetic approach to manipulating fatty acid composition feasible in livestock as well?

There have been many reports of quantitative trait loci (QTL) in farm livestock that affect fat deposition, but there are only a few QTLs known to affect fatty acid composition. A QTL on porcine Chromosome 4 that affects the proportions of linoleic and oleic acids has been described; individual pigs homozygous for the allele from the Iberian

Abbreviations: CLA: conjugated linoleic acid, F: Friesian, *FASN*: fatty acid synthase gene, J: Jersey, L: Limousin, QTL: quantitative trait locus, RSD: residual standard deviation, SNP: single nucleotide polymorphism, SNP2-SNP6: single nucleotide polymorphisms at five positions (coded 2-6) in the *FASN* gene.

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breed had 1.5% less linoleic acid, on average, than those homozygous for the Landrace allele (Perez-Enciso et al. 2000). A later study (Clop et al. 2003) also revealed QTL for a number of traits related to fatty acid composition, including a fatty acid double bond index, an unsaturated fat index, and percentages of linolenic, gadoleic, and myristic acids. Other investigators (Lee et al. 2003) identified two QTLs on porcine Chromosomes 1 and 18 that affected fatty acid composition (linoleic and myristic acids, respectively). A recent study in sheep has identified significant QTLs at the genome-wide level for fatty acid composition in adipose extracted from the *M. semimembranosus* muscle; 12 of 17 fatty acids investigated were affected (Karamichou et al. 2006). In a mapping population of cattle in Texas, Taylor et al. (1998) investigated the growth hormone gene *GH1* on Chromosome 19 as a candidate gene for fat-related traits, including the proportion of unsaturated to saturated fat, and ether-extractable fat percentage, within subcutaneous fat over the *l. dorsi* muscle. The authors recommended that acetyl-CoA carboxylase and fatty acid synthase be tested as candidate genes for fat-related beef carcass traits. Further suggestions for fat-related traits in cattle came from Roy et al. (2005) in relation to fatty acid synthase on Chromosome 19 and mitochondrial glycerol-3-phosphate acyltransferase on Chromosome 26.

The aim of the present study was to investigate genetic differences in fatty acid composition using three resource populations of cattle. Phenotypes were recorded for fatty acid composition in the subcutaneous adipose tissue or milk fat from approximately 4800 cattle, and a screen with microsatellite markers on Chromosome 19 was conducted on a subset of these animals. This report describes the detection of a QTL in beef cattle affecting fatty acid composition of adipose fat, its validation in milk fat in independent populations of dairy cattle, and an investigation of fatty acid synthase as the causative gene for the QTL.

Methods

***J* × *L* backcross (Trial 1).** The trial design involved two *Bos taurus* breeds, Jersey (J) and Limousin (L), extreme for milk production and for muscling, respectively. A reciprocal backcross experiment was performed by mating six first-cross (F_1) bulls (J × L or L × J) to both J and L cows. Three of the F_1 sires were mated to dams in New Zealand, and the other three were mated to dams in Australia. Trial 1 comprises the New Zealand calves; most were generated by artificial insemination, but the 1996 $\frac{3}{4}$ -Limousin

calf crop was produced by embryo transfer using Hereford × Friesian cows as recipients, subsequently calving both single and twin calves. A total of 261 experimental backcross calves (162 $\frac{3}{4}$ -Jersey and 99 $\frac{3}{4}$ -Limousin) were born in Spring 1996, with another 155 born in Spring 1997 (102 $\frac{3}{4}$ -Jersey and 53 $\frac{3}{4}$ -Limousin). These 416 animals (heifers and steers) grazed on pasture and were supplemented with some roughage in winter (no concentrate feeding). Fatty acid percentages were determined (as described below) in adipose tissue sampled from subcutaneous fat collected *post mortem* in 1998 and 1999.

Milk composition trial (Trial 2). A milk composition trial (Trial 2) was performed between November 2000 and February 2001, resulting in the collection of phenotypes during midlactation from approximately 4100 Friesian-sired cows from 21 commercial New Zealand dairy herds. In some cases separate grazing groups were used within a herd and the cow allocation to each of these groups was recorded. All cows were daughters of 11 Friesian sires that were used widely in New Zealand to supply semen for artificial insemination. The cows ranged in age from 2 to 11 years. One pooled milk sample (combining the evening and following morning milk) was collected from each cow in midlactation for the present study.

Milk composition trial (Trial 3). Another milk composition trial (Trial 3) was performed in March 2003 using a single AgResearch herd at "Flock House," Bulls, Manawatu Province, New Zealand, with a total of 248 cows' milk sampled from an evening and the following morning milkings. The animals in this study ranged in age from 2 to 9 years and consisted of approximately equal numbers of Friesian-sired and Jersey-sired cows in late lactation. All cows grazed in one of two grazing groups (both breeds in both grazing groups) on a ryegrass/white clover diet.

Extraction and analysis of fatty acids. In Trial 1 (adipose fat), each fat sample was blended in a food processor and filtered through Whatman 541 filter paper (Whatman International Ltd, United Kingdom) in an oven set at 60°C to separate the fat. The fat layer was then transferred into tubes which were flushed with nitrogen, capped, and stored at -30°C for later analysis. In Trials 2 and 3 (milk samples), 1 ml of 0.15 g/ml EDTA was added to each 40 ml-sample of fresh milk, mixed, and refrigerated overnight. In the morning, samples were centrifuged at 1500 rpm for 4 min. The thick cream layer was

transferred by spatula into 2-ml disposable microfuge centrifuge tubes and centrifuged twice at 15,000 rpm for 3 min, with the fat melted in an oven at 60°C between the centrifugations. The melted fat layer was then transferred into tubes that were flushed with nitrogen, capped, and stored at -30°C for later analysis.

Fatty acids were converted to their methyl esters, and individual fatty acid percentages in each sample were assayed with a gas chromatograph/flame ionization detector. Peak areas on the chromatographs were integrated to quantify the percentages of each fatty acid. The identity of each fatty acid was determined by comparing the retention time of each peak with known standards.

DNA extraction. In Trial 1, DNA was extracted from the sires and the two birth cohorts of calves using blood samples collected in their first year of life, using the method of Montgomery and Sise (1990). The exceptions were the $\frac{3}{4}$ -Limousin calves born in 1996 from which DNA was extracted from ear cartilage using a Qiagen DNeasy Tissue Kit following the manufacturer's instructions. This method was used because of the possibility of blood mixing in twin-born embryo-transfer calves. In Trial 2, DNA samples from 9 of the 11 Friesian sires were obtained from seminal fluid using a standard proteinase-k, phenol/chloroform extraction. DNA samples from all daughters were extracted directly from individual milk samples by standard methods or were isolated from milk blotted onto Whatman FTA paper according to the manufacturer's instructions (Whatman FTA Technology, Whatman International Ltd, United Kingdom). In Trial 3, DNA was extracted from blood samples taken from the cows at the time of their milk sampling for this study, using the method of Montgomery and Sise (1990).

Genotyping of microsatellite markers. For Trial 1, all animals were genotyped for eight microsatellite markers: BM9202, HEL10, BMS2142, BP20, BM17132, BMS1069, ETH3, and BMS601 (Kappes et al. 1997). For Trial 2, the sires and a subset of the cows were genotyped for six microsatellite markers: BM9202, BMS2142, BM17132, BMS1069, ETH3, and BMS601.

Identification of single nucleotide polymorphisms (SNPs). To identify SNP markers, a region of the fatty acid synthase (*FASN*) gene (GenBank accession No. AF285607) from one informative Friesian sire was resequenced. Primers (5'-GGTGCTCGAGGGTGTGGTGCA-3' and 5'-TGCCGGAATT

CCCGCATGGA-3') were designed based on the *FASN* sequence and were used for PCR amplification. Each primer was designed so that the PCR product would have *Xho*I and *Eco*RI restriction endonuclease sites at alternate ends. The PCR fragments (approximately 2 kb in size) were amplified from genomic DNA in a 20- μ l reaction, purified with a High Pure PCR Product Purification Kit (Roche), and digested with *Xho*I and *Eco*RI restriction endonucleases, and the enzymes were inactivated by heating for 20 min at 70°C. The sample was ligated into the plasmid pBluescript KS (Stratagene) and transformed into chemically competent *E. coli* strain XL1-Blue. Individual colonies were analyzed by colony PCR. Plasmid DNA was purified from *E. coli*, and the insert DNA from four individual colonies was sequenced bidirectionally from the T7 and T3 primer sites in the pBluescript plasmid. Typically, each sequencing reaction gave 800-900 bases of reliable sequence. Hence, in total we resequenced approximately 1.8 kb of the bovine *FASN* gene. One exception to this was SNP6, which was identified by direct sequencing of PCR product. The primers used in amplification were 5'-TGCTGTCATGCGGGAAATC-3' and 5'-AACTGCTGCATGAAGAAGCAC-3'. The PCR product was purified with a PCR Product Purification Kit (Roche) and sequenced with a primer with the sequence 5'-GCTCACAATCCACAAGCTG-3'. All sequencing was done with an ABI 3100 DNA sequencer and products from Applied Biosystems (Foster City, CA). The sequences were analyzed by alignment using programs from the Wisconsin Package v10.2 [Genetics Computer Group (GCG), Madison, WI]. Table 1 lists the names given and the descriptions for the polymorphisms identified, consisting of SNPs and a dinucleotide insertion. Assays were designed to genotype these SNPs in the animals from Trials 1-3 via the Sequenom MASSARRAY system. The primers for these assays are also given in Table 1, along with the *FASN* SNP positions, so that their order was SNP4, SNP5, SNP3, SNP2, and SNP6. Haplotypes of sires were determined by using homozygous SNP data in progeny, with flanking markers adding more information.

Statistical analyses. In Trial 1, seven major fatty acids analyzed were myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). In Trials 2 and 3, other fatty acids were also included, namely, those with 4-12 carbons, C4:0, C6:0, C8:0, C10:0, and C12:0. Analyses were performed first using SAS (1995) procedures to determine the important fixed effects in each trial.

Table 1. Summary of single nucleotide polymorphisms (SNPs) identified by resequencing, and the primers used for SNP genotyping

SNP	Position ^a	SNP type	Notes
SNP2	17250 and 17251	AT indel	Intron
SNP3	16907	T to C	exon (silent, leucine)
SNP4	15531	C to A	exon (silent, valine)
SNP5	15603	G to A	intron
SNP6	17924	A to G	exon, nonsynonymous, encoding threonine (ACC) to alanine (GCC)

SNP	Amplification primer 1	Amplification primer 2	Extend primer
SNP2	ACGTTGGATGGCACC GAGTTGAGCCGTGTC	ACGTTGGATGCCGACGG GTGTGCATGTCTG	AGG TTCAGCTGGGTTGCGA
SNP3	ACGTTGGATGGCACC AGTTGAGCCGTGTC	ACGTTGGATGCCGACGG GTGTGCATGTCTG	TGCTCAGGTT CAGCTGGGT
SNP4	ACGTTGGATGGGCGAC CACCGCACGCTG	ACGTTGGATGTGCCGTC GCACACCTGGGAC	TTTGCTGCGCGTTCCTTCTA
SNP5	ACGTTGGATGGGCGAC CACCGCACGCTG	ACGTTGGATGTGCCGTC GCACACCTGGGAC	TGCTGCGCGCCAAGATGG
SNP6	ACGTTGGATGGGCGAC CACCGCACGCTG	ACGTTGGATGTGCCGTC GCACACCTGGGAC	GACTCCAGGCCGCTGCC

^aPosition of each SNP relative to the published cattle sequence of fatty acid synthase (accession AF285607).

For Trial 1, the fatty acid composition data were adjusted for slaughter group (incorporating year of birth and sex combinations) and breed of dam (in combination with herd of origin and birth rank), and adjusted values were calculated in residual standard deviation (RSD) units. After the *FASN* SNPs were genotyped, the combined microsatellite and SNP data were analyzed for linkage on a within-sire basis using QTL Express software (Seaton et al. 2002). The 95% and 99% thresholds were calculated by iterations (1000×), and bootstrapping was used to determine confidence intervals for QTL locations.

For Trial 2, effects were fitted for herd of origin (and associated grazing group), age of cow, Friesian proportion (accounting for crossbreds and grade-up animals, all with a Friesian sire), and a covariate for days-in-milk. Because of the batch processing of milk fat samples in preparation for gas chromatography, an adjustment for methylation batch was also required in the analysis. Adjusted values were generated in RSD units. After analyzing the six microsatellite markers, the *FASN* SNPs were genotyped over a subset of animals (consisting of those cows at the phenotypic extremes for milk fat percentage), and results were analyzed on a within-sire basis using QTL Express software.

A correlation analysis among residuals for 11 fatty acids was performed, followed by a principal components analysis to identify a possible first principal component that might be tested as another trait using QTL Express software.

For Trial 3, breed of cow, age of cow, and grazing group were used to generate standardized residuals;

the covariate for date of calving was found to be nonsignificant. Statistical analyses are described below.

From each trial the SNP data alone (using RSDs) were then analyzed using chi-squared and least-squares procedures (regressing phenotype on genotype), to test the association between a SNP locus (or inherited haplotype from a parent) and a fatty acid component.

Results

Results from the three separate trials that identify and then validate a QTL for fatty acid composition are presented. Resource populations measured for fatty acid composition were those from Trial 1 (using subcutaneous adipose tissue) and Trials 2 and 3 (using milk fat).

Discovery of a QTL for fatty acid composition in adipose tissue (Trial 1). Table 2 shows the overall means, RSDs, and breed-cross comparisons for fatty acid composition in subcutaneous adipose fat from Trial 1. All seven fatty acids analyzed, except myristic and palmitic acids, were affected significantly by breed-cross, with the ¾-Jersey cross having higher values of palmitoleic acid (C16:1) and oleic acid (C18:1), mainly compensated for by lower values of stearic acid (C18:0).

In Trial 1, preliminary analyses detected a QTL on bovine chromosome 19 that significantly affected one fatty acid (C14:0) at the 99% experiment-wise level, using microsatellites and linkage analysis

Table 2. Percentages of the predominant fatty acids in subcutaneous adipose fat in Trial 1: overall means, residual standard deviations (RSD) and breed-cross contrasts \pm standard error

Fatty acid	Overall mean (% of total)	RSD (% of total)	LJJI effect minus LJLL effect (RSD units)	<i>p</i>
C14:0	3.4	0.45	0.08 \pm 0.05	ns
C16:0	23.9	1.20	-0.09 \pm 0.14	ns
C16:1	6.6	1.02	1.18 \pm 0.12	<0.001
C18:0	11.0	2.11	-2.24 \pm 0.24	<0.001
C18:1	42.2	1.89	0.99 \pm 0.22	<0.001
C18:2	1.5	0.17	-0.12 \pm 0.02	<0.001
C18:3	0.7	0.12	-0.07 \pm 0.01	<0.001

LJJI = ¼-Limousin and ¾-Jersey animals; LJLL = ¼-Jersey and ¾-Limousin animals.

(data not shown because more complete data are given in Table 3). Microsatellite BMS1069 was not informative for one of the three sire groups in Trial 1. Three other fatty acids were significant or approached significance at the 95% experiment-wise level (C16:1, C18:0, and C18:1). All QTL that were detected were located at approximately 70 cM.

The *FASN* gene was identified as a positional candidate gene for this QTL because the location of *FASN* on bovine Chromosome 19q22 (Roy et al. 2001, 2005) coincided with the estimated location of the observed QTL, and because variation in *FASN* activity might explain the animal-to-animal variation in fatty acid composition. The cattle *FASN* gene is in approximately the same location as the microsatellite marker IOBT34 (at ~77 cM on the map of Ihara et al. 2004), with marker BMS1069 about 1-2 cM from IOBT34 (Roy et al. 2005). Six SNPs in the cattle *FASN* gene were identified by resequencing and five of these were found subsequently to be polymorphic in our populations (Table 1). The probability values for four fatty acids are shown on a log₁₀ scale in Fig. 1, from the combined analyses of microsatellites and SNPs on Chromosome 19 by position (substituting a *FASN* SNP for microsatellite BMS1069), and the within-sire group results are summarized in Table 3. The QTL effect on C14:0 accounted for a difference of about 0.38% \pm 0.07% of all adipose fat (0.85 RSDs or 11.3% of the C14:0 mean), and the squared corre-

lation coefficient indicated that the QTL accounted for 17% of the total variation in adjusted values for C14:0.

Validation of the QTL in milk (Trial 2). The predominant fatty acids in the milk fat samples of Trial 2 were C16:0 30.5% (RSD = 2.7%), C18:1 15.7% (1.8%), C14:0 12.6% (0.8%), and C18:0 9.5% (1.3%). The most significant linkage results using both microsatellites and SNPs combined are given in Table 4 (six of these fatty acids were significant at $p < 0.01$ and four at $p < 0.05$). The probability values for the six most significant fatty acids for one Friesian sire, Sire A, are shown on a log₁₀ scale in Fig. 2, from the combined analyses of microsatellites and SNPs on Chromosome 19 by position; microsatellites BMS1069 and ETH3 were not informative for this sire. A QTL was identified that affected C10:0, C12:0, C8:0, C14:0, C18:2, and C18:1 (in order of significance), within the progeny of Sire A. The estimated location of this milk fat QTL coincided with the QTL observed for adipose fat in Trial 1.

In Trial 2, a correlation analysis among residuals was performed for the ten fatty acids that had a significant QTL. The largest positive correlations were as follows: C6:0 \times C8:0, 0.70; C8:0 \times C10:0, 0.83; C10:0 \times C12:0, 0.96; C12:0 \times C14:0, 0.76; C8:0 \times C12:0, 0.70; C10:0 \times C14:0, 0.74; and C18:1 trans \times CLA, 0.78. The two most negative correlations were between C12:0 and C18:1 (-0.54), and C14:0 and C18:1 (-0.51). A

Table 3. QTL on Chromosome 19 for fatty acid composition in subcutaneous adipose fat for individual sire-progeny groups in Trial 1 showing the chromosomal location and confidence interval at the maximal *F* statistic (with threshold), the size of the estimated additive effect of the QTL and its standard error (RSD units), and *R*² (squared correlation coefficient at the location)

Trait	Sire group	Location (range) (cM)	<i>F</i> stat (0.95) ^a	Size of effect (\pm SE) in RSD units	<i>R</i> ²
C14:0	402	76 (68–108)	26.9 (11.9) ^b	0.85 (0.16)	0.17
C16:1	402	70 (46–108)	12.0 (14.1)	0.62 (0.18)	0.07
C18:0	417	66 (59–108)	11.1 (12.4)	-0.68 (0.20)	0.09
C18:1	394	72 (16–77)	15.3 (15.2)	0.77 (0.20)	0.08

^aThe threshold *F* statistic is given in parentheses for an 0.95 experiment-wise significance.

^b0.99 experiment-wise threshold for C14:0 = 15.5.

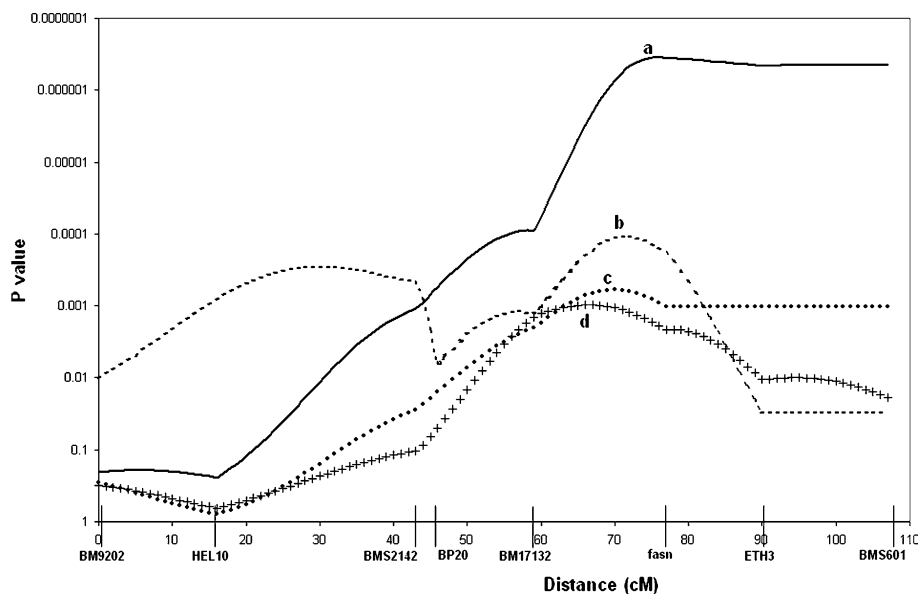


Fig. 1. Log_{10} -transformed probabilities for fatty acid composition traits in adipose fat across Chromosome 19 within sire groups (Trial 1). Fatty acids were (a) residuals for C14:0 for sire group 402 (—); (b) residuals for C18:1 for sire group 394 (- - -); (c) residuals for C16:1 for sire group 402 (· · · ·); (d) residuals for C18:0 for sire group 417 (+ + +).

principal components analysis showed that the first principal component accounted for 37% of the variance among the fatty acids, the second a further 18%, and the third a further 15%. The first principal component was used as a variate to plot a new QTL map and this gave a very similar plot to that for C10:0 in position and shape in Fig. 2. The maximum height was slightly greater for this variate than for C10:0 ($p = 2.53\text{E-}09$ and $p = 7.92\text{E-}09$, respectively, i.e., a smaller probability for the first principal component).

Linkage disequilibrium analysis

Trial 1. Analysis of SNPs in Trial 1 animals led to the identification of a series of five-SNP haplotypes. The three sires in Trial 1 had a combined total of three haplotypes for SNP2-SNP6 (Table 5). Using

SNP and informative microsatellite flanking markers together, the numbers of animals with each haplotype are also given by sire in Table 5. Each sire carried the haplotype CGCDA (ordered by nucleotide sequence from their positions in Table 1 as SNP4, SNP5, SNP3, SNP2, and SNP6, respectively; D = deletion) from the Limousin parent, and the paired chromosome from the Jersey parent carried one of two haplotypes, either the CGTIG or the AATIG nucleotide sequence (I = insertion). Numbers of progeny receiving the two haplotypes from sire 394 appeared to be imbalanced because only the third, fourth, and fifth SNPs (i.e., SNP3, SNP2, and SNP6, respectively) of the haplotype were informative. Also, the high level of heterozygosity from the sire-394 haplotype CGCDA, when paired with chromosomes from Jersey cows carrying

Table 4. QTL on Chromosome 19 for fatty acid composition in milk fat among daughters of one Friesian sire in Trial 2 showing the chromosomal location and confidence interval at the maximal F statistic (with thresholds), the size of the estimated additive effect of the QTL and its standard error (RSD units), and R^2 (squared correlation coefficient at location)

Trait	Location (range) (cM)	F stat ^a	(0.95, 0.99)	Size of effect (\pm SE) in RSD units	R^2
C6:0	68 (59–77)	9.1	(7.1 9.8)	–0.46 (0.15)	0.04
C8:0	71 (68–77)	26.1	(6.4 10.0)	–0.86 (0.17)	0.11
C10:0	71 (68–77)	36.4	(6.7 10.0)	–1.07 (0.18)	0.15
C12:0	71 (68–77)	33.2	(6.3 9.8)	–1.07 (0.19)	0.14
C14:0	68 (51–77)	21.2	(6.5 9.6)	–0.74 (0.16)	0.09
C18:0	75 (11–93)	6.7	(6.6 9.1)	0.45 (0.17)	0.03
C18:1	72 (43–93)	11.9	(6.6 10.2)	0.80 (0.23)	0.05
C18:1 trans	51 (22–93)	9.5	(7.3 10.8)	0.54 (0.18)	0.04
C18:2	74 (51–93)	18.1	(7.0 10.9)	0.74 (0.17)	0.08
C18:2 (CLA)	51 (22–108)	9.7	(6.9 10.6)	0.52 (0.17)	0.04
C18:3	78 (0–93)	6.0	(6.8 10.6)	0.42 (0.17)	0.03

CLA = conjugated linoleic acid.

^aThe threshold F statistics are given in parentheses for 0.95 and 0.99 experiment-wise significances.

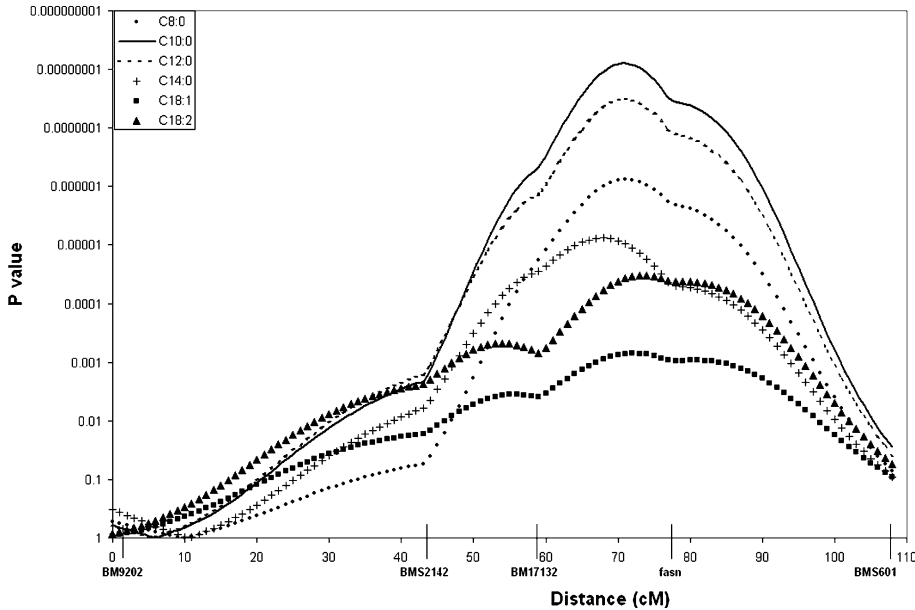


Fig. 2. Log₁₀-transformed probabilities for fatty acid composition traits in milk fat across Chromosome 19 for Friesian Sire A (Trial 2).

mainly the CGTIG or the AATIG haplotype, meant that the haplotype inherited from the sire could not be derived. The haplotype inherited from the dam of each backcross calf in Trial 1 was determined by subtraction. There was a predominance of CGTIG haplotypes among dams, with 138 progeny (144 when including information from flanking markers), followed by the AATIG haplotype with 35 (50) progeny, and then the CGCDA haplotype with 15 (39) progeny.

The haplotype difference in C14:0 fatty acid percentage in subcutaneous adipose fat (Table 6, top panel) was highly significant ($p < 10^{-6}$) for sire 402, and it approached significance in the same direction for sire 417 with the same haplotype comparison ($p = 0.07$). The haplotype effect, CGCDA minus CGTIG, was also significant for sire 394 ($p = 0.02$), but it was in the opposite direction from the contrast of CGCDA minus AATIG. Sire 394 was homozygous for SNP4 and SNP5, so the contrast examined may effectively have represented another haplotype.

Table 6 also shows results from sire and dam haplotype effects fitted together [second panel, with fewer animals than for sire effects (top panel) because there were some animals with unknown dam haplotype], along with the sire and dam SNP4 effects fitted together (bottom panel). Results for SNP4 and SNP5 were identical because of their very close linkage. The size of the effect of nucleotide substitution C minus A at SNP4 for sire 402 was 0.75 ± 0.18 RSD, or $0.34\% \pm 0.08\%$ of all fat (i.e., 9.9% of the mean for C14:0 in adipose fat). The size of the effect for sire 417 was about half of that for sire 402, and in the same direction. The sign of the C-minus-A substitution in independent dams was the same as in the sires, and it was significant among the Jersey dams ($p = 0.008$).

Trial 2. The same five SNPs and the haplotypes for *FASN* were tested for an association with milk fat phenotype in Trial 2 cows. Table 7 (top panel) shows the results for association of all cows genotyped, combining daughters of Sire A and other available

Table 5. Summary of sire haplotypes in Trial 1 showing numbers of progeny with sire-derived haplotypes

Sire family	Haplotypes (SNP4_5_3_2_6) ^a	Number of progeny	Number of progeny using flanking markers ^b
394	CGCDA	15	36
	CGTIG	50	67
402	CGCDA	51	61
	AATIG	44	79
417	CGCDA	41	45
	AATIG	43	51

^aD = deletion (2-base); I = insertion.

^bTotal progeny numbers when including flanking markers for classification.

Table 6. Effects of haplotype in Trial 1 (SNP4, SNP5, SNP3, SNP2, SNP6, including additional data from informative flanking markers) on C14:0 percentage in subcutaneous adipose fat (results expressed as standardized residuals), with probability *p* (*t* test)

Family	Test	Difference	<i>p</i>
Sire haplotype alone			
Sire 394	CGCDA–CGTIG	−0.44 ± 0.19	0.02
Sire 402	CGCDA–AATIG	0.82 ± 0.17	10 ^{−6}
Sire 417	CGCDA–AATIG	0.36 ± 0.20	0.07
Sire and dam haplotypes fitted			
Sire 394	CGCDA–CGTIG	−0.49 ± 0.26	0.06
Sire 402	CGCDA–AATIG	0.74 ± 0.19	0.0002
Sire 417	CGCDA–AATIG	0.40 ± 0.22	0.07
Jersey dam haplotypes	CGCDA–AATIG	0.44 ± 0.33	0.18
Limousin dam haplotypes	CGCDA–AATIG	0.05 ± 0.27	0.85
Sire and dam SNP4 fitted ^a			
Sire 402	C–A	0.75 ± 0.18	7 × 10 ^{−5}
Sire 417	C–A	0.37 ± 0.20	0.07
Jersey dams	C–A	0.46 ± 0.17	0.008
Limousin dams	C–A	0.15 ± 0.20	0.47

^aSire 394 homozygous.

Friesian sires. SNP4 and SNP5 effects were both significant ($p < 0.05$), with effects on C14:0 percentage of -0.28 ± 0.14 RSD units (for CC minus AA) or -0.33 ± 0.14 RSD units (for GG minus AA), respectively. In the largest Friesian sire group evaluated in Trial 2 (sire A, carrying haplotypes CGCDA and AATIG), 111 daughters inherited the CGCDA haplotype from the sire, and 107 daughters inherited the AATIG haplotype. The difference in C14:0 percentage in milk fat between the haplotypes CGCDA and AATIG was significant ($p < 0.001$), with an effect of -0.42 ± 0.13 RSD units (Table 7, bottom panel). Fatty acids C6:0, C8:0, C10:0, C12:0, and C18:1 were also affected by these sire haplotypes at similar or more significant probabilities to C14:0 (Table 7) and with similar sizes of genotypic effects (on an RSD basis). As was found in Trial 1, the effect on C14:0 was in the opposite direction of the effect on C18:1.

Trial 3. The *FASN* SNPs were further validated for the milk fat phenotype by genotyping the Trial 3 cows. The predominant fatty acids found in milk fat from Friesian-sired and Jersey-sired cows in this trial were C16:0 29.3% (RSD = 2.1%), C18:1 19.1% (2.3%), C14:0 10.1% (0.9%), and C18:0 10.1% (1.4%). Overall, 94% of the 121 Friesian-sired cows and 68% of the 113 Jersey-sired cows whose DNA was analyzed were purebred or $\frac{3}{4}$ bred. Table 8 shows the genotype numbers and allele frequencies for the five SNPs in each breed.

Over both breeds combined (data not tabulated), SNP3 was associated with C18:0 percentage ($p < 0.05$), while SNP4 and SNP5 were both significant for percentages of C14:0 and of C18:1 (all $p < 0.05$). When analyzing within breed (Table 9), only the Jersey data were significant, as follows: the genotype effect (CC minus AC) on C14:0 percentage in milk fat was

Table 7. Association tests for five SNPs (2-6) and a Sire A-derived haplotype test ($n = 218$ cows) for the presence of C14:0 and five other fatty acids in milk fat in Trial 2 (results expressed as standardized residuals), with probability *p* (*t* test)

Fatty acid	Genotype	Difference	<i>P</i>
Cow SNP test			
C14:0	SNP2 ^a (DD – II)	−0.38 ± 0.20	0.06
	SNP3 (CC – TT)	−0.32 ± 0.21	0.12
	SNP4 (CC – AA)	−0.28 ± 0.14	0.04
	SNP5 (GG – AA)	−0.33 ± 0.14	0.02
	SNP6 (AA – GG)	−0.09 ± 0.15	0.54
Sire haplotype test ^b			
C6:0		−0.35 ± 0.12	0.004
C8:0		−0.54 ± 0.13	<0.0001
C10:0		−0.57 ± 0.14	<0.0001
C12:0		−0.55 ± 0.14	0.0002
C14:0		−0.42 ± 0.13	0.001
C18:1		0.52 ± 0.16	0.001

^aSome SNPs were run in only one family (Sire A), while others were run for progeny of Sire A and other sires in the Trial.

^bCGCDA effect minus AATIG effect.

Table 8. Genotype numbers and allele frequencies in the Friesian and Jersey breeds for five SNPs in Trial 3

Breed	Genotype	SNP4	SNP5	SNP3	SNP2 ^a	SNP6
Genotype numbers						
Friesian	11 ^b	21	20	17	47	15
	12	68	62	60	64	51
	22	37	35	46	14	63
Jersey	11	8	9	1	81	1
	12	47	40	30	32	28
	22	57	56	80	1	83
Allele frequencies						
Allele described ^b		A	A	C	I	A
Friesian		0.44	0.44	0.38	0.63	0.31
Jersey		0.28	0.28	0.14	0.85	0.13

^aI = 2-base insertion for SNP2.

^bThe allele described as "1" is named in the second panel.

-0.64 ± 0.19 RSD units ($p = 0.0009$) for SNP4, and the genotype effect (GG minus AG) on the same trait was -0.70 ± 0.20 RSD units ($p = 0.0007$) for SNP5. SNP4 and SNP5 in Jerseys had opposite effects on C18:1 compared with their effects on C14:0, i.e., respectively, 0.56 ± 0.19 RSD units ($p = 0.0046$) and 0.62 ± 0.20 RSD units ($p = 0.0027$) for C18:1. Consequently, an allelic substitution at SNP4 (A instead of C) or SNP5 (A instead of G) led to an increase in the C14:0 percentage in milk fat and a corresponding reduction in the C18:1 percentage in milk (RSD units). Converting RSD units back to the units of measurement, a SNP4 or SNP5 substitution led to a 2.8-fold greater change in C18:1 concentration than in C14:0 concentration. The same trends appeared to apply to Friesians as to Jerseys, although contrasts were not significant in Friesians.

Discussion

The aim of this study was to identify QTL that explain some of the genetic variation in fatty acid composition in cattle. Initially, we conducted a screen of Chromosome 19 using a reciprocal Jersey × Limousin backcross (Trial 1), with phenotypes for

subcutaneous adipose fatty acid composition. A significant QTL was identified for two fatty acids (Table 3) on Chromosome 19, and a candidate gene for this QTL was identified as *FASN*, a central multifunctional enzyme consisting of seven distinct catalytic sites and responsible for *de novo* fatty acid biosynthesis (Roy et al. 2001). Genetic variants of *FASN* have been reported recently to be associated with fat percentage in bovine milk (Roy et al. 2006). Using different resource populations, the present study shows that *FASN*, or possibly a closely linked gene, affects adipose fat composition and milk fat composition. The significant QTL effects in milk fat were also found to extend to short-chain fatty acids (C6:0, C8:0, C10:0, and C12:0), which do not exist in adipose fat.

Having identified a QTL on Chromosome 19 by using microsatellites and having posited a candidate gene, a haplotype from a set of five SNPs from the *FASN* gene was then tested for both linkage and association, and the haplotype results are shown in Table 6 for Trial 1. Significant results are shown for both sire and dam effects. Two SNPs in particular, SNP4 and SNP5, showed large effects (up to 0.8 RSD units, with the power of the design sufficient to

Table 9. SNP4 and SNP5 effects on percentages of C14:0 and C18:1 fatty acid in milk fat from Friesian-sired (F) and Jersey-sired (J) cows in Trial 3

	SNP ^a	n	p		Allele differences
C14:0					
F-sired	SNP4	118	0.63	CC-AC	-0.20 ± 0.21 ($p = 0.35$)
	SNP5	110	0.82	GG-AG	-0.11 ± 0.22 ($p = 0.62$)
J-sired	SNP4	111	0.0041	CC-AC	-0.64 ± 0.19 ($p = 0.0009$)
	SNP5	104	0.0031	GG-AG	-0.70 ± 0.20 ($p = 0.0007$)
C18:1					
F-sired	SNP4	118	0.53	CC-AC	0.24 ± 0.21 ($p = 0.26$)
	SNP5	110	0.76	GG-AG	0.12 ± 0.22 ($p = 0.59$)
J-sired	SNP4	111	0.015	CC-AC	0.56 ± 0.19 ($p = 0.0046$)
	SNP5	104	0.0082	GG-AG	0.62 ± 0.20 ($p = 0.0027$)

Least-squares means in units of standardized residuals.

^aNo SNP tests in the within-breed analyses of SNP2, SNP3, or SNP6 were significant.

reach statistical significance with an effect of about 0.4 RSD or more).

It should be noted that of the SNPs discovered in the *FASN* gene herein, only SNP6 encodes an amino acid change (Table 1). As the other SNPs are unlikely to affect the enzyme activity of the fatty acid synthase protein or the expression of the *FASN* gene, the most likely scenario is that SNP4 and SNP5 are in strong linkage disequilibrium with the true functional variant. However, there are no other obvious candidate genes in the immediate vicinity or other potentially causative SNPs in the region.

From the within-sire analyses (Trial 1, Table 3; and Trial 2, Table 4), we showed that the confidence intervals were very wide, often up to 50 cM. Accordingly, from the sire families alone, there was no way of distinguishing between the genes *FASN* and *GH1*. However, from the SNP data, the likely interval for strong linkage disequilibrium is acknowledged as less than 0.3–0.5 cM for cattle breeds such as the Holstein-Friesian and Jersey (Goddard et al. 2006; Spelman and Coppieters 2006). Because the mapped distance between *FASN* and *GH1* is 3.8 cM, it would be unlikely that *FASN* SNPs are in significant linkage disequilibrium if the real gene was *GH1*. In addition, we analyzed *FASN* SNP4 data from *dams* in Trial 1 (Table 6), and the effect from Jersey dams was also significant. Strong linkage disequilibrium is also unlikely to be observed consistently across breeds (Trials) at this distance.

The same five SNPs were also tested in two cattle resource populations with milk fat phenotypes to validate the linkage and association with the SNPs. SNP4 and SNP5 were again found to show associations with C14:0 percentage in milk fat. They were consistent in the direction of the effects in milk fat for both SNP4 and SNP5 across Trial 2 (Table 7; a significant effect in Friesians) and Trial 3 (Table 9; a significant effect in Jerseys, with Friesians in Trial 3 carrying the same sign of effect, although not significant). However, an important finding was that the SNP allelic effects on C14:0 percentage in the milk fat of Trials 2 and 3 (Tables 7 and 9) were opposite in sign from those found for C14:0 percentage in adipose fat in Trial 1 (Table 6). The "A" alleles of both SNP4 and SNP5 were associated with increased percentages of C14:0 in milk fat, but they had the opposite effect in adipose fat. This was probably a tissue expression effect, not a breed effect, because the Jersey breed was common to Trials 1 and 3.

The *FASN* protein in adipose tissue synthesizes mainly C14:0 and C16:0, whereas *FASN* in mammary tissue synthesizes fatty acids of chain length varying from C4:0 to C16:0 in 2-carbon increments. The thioesterase enzymes responsible for cleaving

off the acyl groups from the *FASN* complex are different in bovine mammary *FASN* and adipose *FASN* (Smith 1994), although this is not well characterized. A possible mechanism for *FASN* is that SNP4 and/or SNP5 may be associated with thioesterase specificity for fatty acids, and this association might also be tissue specific. The level of regulation of *FASN* by external factors is different in adipose and mammary tissue, and this difference may be responsible for the different SNP effects on C14:0 in the two tissue types. For example, the *FASN* gene activity *in situ* is negatively regulated by C18:1 and other long-chain fatty acids (Hansen and Knudsen 1987; Gaynor et al. 1995), and C18:1 percentages are much higher (by a factor of ~2.5) in adipose tissue than in milk fat.

Microsatellites on Chromosome 19 were significantly associated with C14:0 concentrations but not with C16:0 concentrations, in both adipose and milk fat. The association was also observed for C6:0, C8:0, C10:0, and C12:0 in milk. One explanation for this might be that the effect observed for C16:0 is confounded by contributions from dietary fatty acids, whereas dietary input for C6:0, C8:0, C10:0, C12:0, and C14:0 is less.

Conclusions

A QTL was identified for a series of fatty acids in adipose fat and milk fat on bovine Chromosome 19, and *FASN* was postulated as a candidate gene for the QTL. Five SNPs on *FASN* were significantly associated with fatty acid percentages, up to 0.8 RSD in magnitude, and the SNP4 or SNP5 substitution effects for C14:0 percentage were found to have an effect in the opposite direction in adipose fat than that of milk fat.

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