Sterol regulatory element binding transcription factor 1 (*SREBF1*) polymorphism and milk fatty acid composition

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ABSTRACT

Milk is known to contain high concentrations of saturated fatty acids—such as palmitic (16:0), myristic (14:0), and lauric (12:0) acids—that can raise plasma cholesterol in humans, making their presence in milk undesirable. The main objective of our candidate gene study was to develop genetic markers that can be used to improve the healthfulness of bovine milk. The sterol regulatory element binding transcription factor 1 (*SREBF1*) known to regulate the transcription of lipogenic genes together with SREBF chaperone and insulin induced gene 1 were the candidate genes. The results showed significant association of the overall *SREBF1* haplotypes with milk production and variations in lauric (12:0) and myristic (14:0) acid concentrations in milk. Haplotype H1 of *SREBF1* was the most desirable to improve milk healthfulness because it was significantly associated with lower lauric (12:0) and myristic (14:0) acid concentrations compared with haplotype H3 of *SREBF1*, and lower lauric acid (12:0) concentration compared with haplotype H2 of *SREBF1*. Haplotype H1 of *SREBF1*, however, was significantly associated with lower milk production compared with haplotype H3 of *SREBF1*. We did not detect any significant associations between genetic polymorphisms in insulin induced gene 1 (*INSIG1*) and SREBF chaperone and milk fatty acid composition. In conclusion, genetic polymorphisms in *SREBF1* can be used to develop genetic tools for the selection of animals producing milk with healthier fatty acid composition.

Key words: milk fatty acid, sterol regulatory element binding transcription protein 1 (SREBP1), candidate gene, dairy cow

INTRODUCTION

Dietary SFA are known to increase plasma total cholesterol and low-density lipoprotein cholesterol concentrations in humans, leading to increased risk of developing cardiovascular diseases (Kris-Etherton and Yu, 1997). Not all SFA, however, have the same effect on plasma cholesterol concentrations. Palmitic (16:0) and myristic (14:0) acids, for example, can increase plasma cholesterol concentrations much more compared with other SFA, and, therefore, their presence at high concentrations in human diets is undesirable (Kris-Etherton and Yu, 1997). The MUFA, however, are considered neutral with regard to plasma cholesterol concentrations, and their presence in human diets can be increased at the expense of SFA (Clarke et al., 1997; Kris-Etherton and Yu, 1997; Schaefer, 2002). In recent years, several studies researched opportunities for decreasing the concentration of atherogenic FA in bovine milk, by dietary and genetic means, to improve the healthfulness of milk (Jensen, 2002; German and Dillard, 2006; Roy et al., 2006; Soyeurt et al., 2006; Schennink et al., 2007; Schennink et al., 2009; Bouwman et al., 2011; Rincon et al., 2012). The genetic approach is more plausible than the dietary approach because conversion of dietary unsaturated fatty acids (UFA) into milk UFA in ruminants is hampered by ruminal biohydrogenation (Doreau and Chilliard, 1997). The advantage of genetic improvement of milk healthfulness is supported by moderate to high heritabilities of milk FA (Bobe et al., 2008; Stoop et al., 2008; Arnould and Soyeurt, 2009; Bastin et al., 2011; Bouwman et al., 2011). We designed a study to provide tools for the selection of animals with healthier milk FA composition based on the candidate gene approach to discover genetic polymorphisms that can be used as genetic markers in the selection process. The candidate genes chosen for our study were the sterol regulatory element binding transcription factor 1 (*SREBF1*)—known to activate the expression of lipogenic genes such as acetyl-CoA carboxylase α , fatty acid synthase (*FASN*), ELOVL fatty acid elongase 6,

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and stearoyl-CoA desaturase (Horton et al., 2002)—together with the insulin induced gene 1 (*INSIG1*) and SREBF chaperone (*SCAP*) that are required for the sterol regulatory element binding protein (*SREBP*)-1c proteolytic activation.

The *SREBP*-1c transcription factor is encoded by the *SREBF1* gene (Horton et al., 2002). In an active conformation, *SCAP* transports *SREBP*-1c from endoplasmic reticulum (**ER**) to the Golgi apparatus, where the N-terminal domain of *SREBP*-1c is released into the cytosol after a sequence of proteolytic cleavages (Brown and Goldstein, 1997, 2009). The cleaved *SREBP*-1c then enters the nucleus where it activates the transcription of genes from the FA biosynthetic pathways. When *SCAP* is in an inactive conformation, it prevents *SREBP*-1c transport to the Golgi apparatus. As a result, the N-terminal domain of *SREBP*-1c cannot be released and no transcriptional activation of sterol regulatory element-containing genes can occur. Another protein, *INSIG1*, was found to reside in the ER membrane and to bind to *SCAP* in the presence of oxysterols, leading to inhibition of *SCAP*-*SREBP*-1c transport to the Golgi apparatus despite *SCAP* being in an active conformation (Brown and Goldstein, 1997, 2009).

We hypothesized that some of the differences in milk FA composition in dairy cattle are caused by polymorphisms in the lipogenesis regulatory gene *SREBF1*, or by polymorphisms in *SCAP* and *INSIG1* genes that regulate posttranslational activation of *SREBF1* gene product, or both. The *SREBF1*, *INSIG1*, and *SCAP* genes were sequenced to identify SNP. After genotyping animals for the discovered SNP, intragenic haplotypes were reconstructed and the associations between the haplotypes and milk FA composition were tested for each gene separately. The results of the association tests are presented in this paper.

MATERIALS AND METHODS

Animals and Milk FA Analysis

Milk samples were collected during morning milking once a month throughout a 305-d lactation period and stored at -20°C until further analysis. The number of milk samples used in the analysis was 3,510, 3,693, and 3,757 for *SREBF1*, *INSIG1*, and *SCAP*, respectively, and the number of cows from which the samples were collected was 430, 455, and 463, respectively. Out of the total number of animals in *SREBF1*, *INSIG1*, and *SCAP* data sets, respectively, 300, 301, and 302 cows, daughters of 83, 84, and 85 sires, were from the Iowa State University dairy herd; 130, 154, and 161 cows, daughters of 89, 107, and 107 sires, were from the Kan-

sas State University dairy herd. Only 4 sires were used in both herds. All the animals were treated in accordance with guidelines established by the Iowa and Kansas State University Committees on Animal Care. The extraction of milk FA with subsequent esterification into methyl esters for analysis by gas chromatography was performed according to the procedure published by Chouinard et al. (1999) with minor modifications. The procedure is based on the original milk lipid extraction method with hexane and isopropanol developed by Hara and Radin (1978) and FA methyl ester production method developed by Christie and Clapperton (1982). After thawing, milk samples were mixed by vortex for 10 s, and 25-mL aliquots were transferred to 50-mL Teflon centrifuge tubes prerinsed with chloroform/methanol (2/1 vol/vol). Following centrifugation at $17,800 \times g$ for 30 min at 4°C , the liquid phase of the milk samples was removed and 18 mL of hexane/isopropanol (3/2 vol/vol) was added for every 1 g of lipids with subsequent vortexing for 1 min. To achieve a better separation of hexane phase containing lipids from water phase containing proteins, 12 mL of 6.7% sodium sulfate (Na_2SO_4) solution was added for every 1 g of lipids and samples were vortexed for 1 min. After centrifugation at $2,500 \times g$ for 5 min at 4°C , the hexane fraction containing lipids was removed into a scintillation vial prerinsed with chloroform/methanol (2/1 vol/vol) and an aliquot containing 40 mg of lipids (400 μL) was transferred into an esterification vial. After the evaporation of hexane under nitrogen gas, 2 mL of hexane was added to the esterification vial followed by 40 μL of methyl acetate and vortexed for 30 s. The methylation step was initiated by the addition of 40 μL of sodium methoxide (0.4 mL of 5.4 M sodium methoxide in 1.75 mL of methanol) solution. The mixture was vortexed for 30 s and allowed to react for 10 min at room temperature. The reaction was terminated by the addition of 60 μL of oxalic acid (1 g of oxalic acid/30 mL of diethyl ether) solution. The samples were centrifuged at $2,400 \times g$ for 5 min at 4°C , and a clear hexane fraction that contained FA methyl esters was placed into gas chromatography vials, purged with nitrogen gas for 3 s, capped, and used for chromatographic analysis. In the above steps, capping of the vials that contained hexane lipid extract was preceded by purging with nitrogen gas for 3 s.

A Varian 3900 gas chromatograph (Varian Analytical Instruments, Walnut Creek, CA) equipped with CP-8400 auto-sampler, CP-8410 auto-injector, CP-1177 split/splitless injector, and flame ionization detector was used to analyze FA methyl esters with helium as a carrier gas. One microliter of FA methyl esters in hexane was injected (split ratio 50:1) into a fused silica capillary column (Supelco-2560 Capillary Column, Sig-

ma-Aldrich, St. Louis, MO; 100 m × 0.25 mm i.d., with 0.2 µm film thickness) with the initial column temperature set at 70°C and held at 70°C for 4 min. Then, the column temperature was increased to 175°C, at the rate of 13°C/min, and held at 175°C for 27 min with a subsequent increase to 215°C, at the rate of 4°C/min, where it was held for 28 min (Sehat et al. 1998). The injector and detector temperatures were set constant at 220°C. Peak area was measured by integration using Star Chromatography Workstation Version 6 (Varian Analytical Instruments), and peaks were identified by comparing the retention times with separately run FA methyl ester standards (Matreya LLC, Pleasant Gap, PA).

The concentrations of 39 milk FA were analyzed, but data for only 16 FA were used in the association analysis because of the low concentrations of remaining FA. The C14 [C14 desaturation index = 14:1/(14:0 + 14:1)], C16 [C16 desaturation index = 16:1/(16:0 + 16:1)], and C18 [C18 desaturation index = 18:1/(18:0 + 18:1)] desaturation indices and elongation index [elongation index = (18:0 + 18:1)/(16:0 + 16:1 + 18:0 + 18:1)] were used in association analysis to test for the differences in desaturation and elongation of certain FA. The contributions of individual FA to atherogenic potential for a lipid source or a diet are summarized by an atherogenic index that was developed by Ulbricht and Southgate (1991). The atherogenic index, described by these authors, ranks mixtures of FA according to their propensity to cause atherogenesis, as predicted from concentrations of individual FA in the dietary lipid. The atherogenic index = [12:0 + 4(14:0) + 16:0]/(ΣMUFA + ΣPUFA), and it was used in association analysis to test for differences in the atherogenic potential of milk.

SNP Discovery and Genotyping and Protein Sequence Alignment

Genomic DNA was extracted from the white blood cells of a dairy cow using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA) according to the instructions. The *SREBF1*, *INSIG11*, and *SCAP* (Supplemental Table S1, available online at <http://www.journalofdairyscience.org/>), were sequenced in exonic and flanking intronic regions to discover SNP. A set of 12 DNA samples from cows that were daughters of 12 different sires was used for the SNP discovery. The PCR primers were designed with Primer3 (version 0.4.0) software (Rozen and Skaletsky, 2000). Conventional or hot-start PCR was performed to amplify the DNA regions of interest. The PCR reaction mixtures were prepared according to the manufacturer's instructions with a total reaction volume of 25 µL containing

50 ng of genomic DNA. The PCR was performed in a DNA Engine thermal cycler (Bio-Rad Laboratories, Hercules, CA) using an optimal annealing temperature for a particular primer set (Supplemental Table S2, available online at <http://www.journalofdairyscience.org/>) that was determined by a PCR temperature gradient. Before sequencing, the PCR product was cleaned up from unused primers and deoxynucleotide triphosphates with ExoSAP-IT (USB Corporation, Cleveland, OH) that contained exonuclease 1 and shrimp alkaline phosphatase (Hanke and Wink, 1994). To identify SNP, reverse and forward sequences representing 12 different samples were aligned using Vector NTI Advance 10 (Life Technologies, Grand Island, NY). Genotyping of discovered SNP was performed with Sequenom MassARRAY platform (Sequenom Inc., San Diego, CA) using 10 ng of genomic DNA dissolved in DNase-free water (Gabriel et al., 2009). Haplotypes and their frequencies were estimated using PHASE (version 2.1) program (Stephens et al., 2001; Stephens and Donnelly, 2003). Only samples with probability ≥0.9 for the best haplotype pair were used in the analysis. Multiple alignment of SREBP1 protein sequences from mammalian species was performed using Clustal X version 2.0 (Larkin et al., 2007).

Statistical Analysis

A linear mixed model for longitudinal data was used to perform statistical analysis with ASReml (Gilmour et al., 2009). The haplotype substitution model used to test the association between intragenic haplotypes and milk FA composition was the following:

$$Y_{ijkn} = \mu + \text{dim}_i + \text{dim}_i^2 + \text{dim}_i^3 + \text{cg}_j + \text{dim}_i \times \text{cg}_j + \text{dim}_i^2 \times \text{cg}_j + \text{dim}_i^3 \times \text{cg}_j + \sum b_k H_k + A_n + \varepsilon_{ijkn},$$

where Y is the response variable; μ is the general mean; dim , dim^2 , and dim^3 are covariates describing linear, quadratic, and cubic effects of DIM on milk FA composition; cg is a fixed effect of contemporary group (= 24 classes) created by combining herd (2 herds), season of calving (4 seasons: December to February, March to May, June to August, and September to November), and age of calving (3 age groups: group 1 = <2.2 yr old, group 2 = between 2.2 and 4 yr old, and group 3 = >4 yr old) effects; H_k is a haplotype effect fitted as a covariate coded as 0, 1, or 2 for the number of copies of each haplotype present in an animal; b_k is the partial regression coefficient that is the haplotype substitution effect for the k th haplotype as a deviation from the effect of the most frequent haplotype set to zero

to have a full rank model; A_n is the random additive genetic effect of n 's animal; and ε is a residual error. The correlations between repeated measures taken on the same animal were modeled using the first-order autoregressive covariance structure that assumes equal variances and correlations that decline exponentially with an increase in the distance between time points (Littell et al., 1998) because milk samples were collected repeatedly for the same animal throughout 305-d lactation period. The covariance structure was selected based on the biology of the experiment and using Akaike's and Bayesian information criteria and the residual log-likelihood (Sawalha et al., 2005). The higher order terms for DIM were introduced to account for a nonlinear trend of the response variables over time. The cubic term of DIM allows the response variable to have different slopes whenever the direction of its trend changes over time. Haplotypes with a population frequency more than 0.05 were used in the analysis and haplotypes with a population frequency less than 0.05 were pooled into the "other" category. The random additive animal genetic effects were fitted into the statistical model using inverse of an additive genetic relationship matrix that was constructed from a pedigree file containing information about sire, dam, paternal and maternal grandsires and granddams for each cow on the study. Adjusted P -values for the 2-stage Benjamini and Hochberg step-up false discovery rate-controlling procedure (Benjamini et al., 2006) that amended the original linear step-up procedure of Benjamini and Hochberg, (1995) were calculated using Bioconductor multtest package in R (Pollard et al., 2011) to account for testing 3 genes at the same time and when F-tests for the overall haplotype effects were considered. The significance of haplotype effects was declared if adjusted $P < 0.05$. If the F-tests were considered significant, the pairwise comparisons between all haplotypes within each gene were performed and estimates of the differences between haplotype effects, their standard errors, and P -values were reported. The Bonferroni adjustment was used to control type I error rate during multiple comparisons between different haplotype effects within *SREBF1* and significance was declared if the P -value was ≤ 0.0167 (0.05 divided by the number of haplotype comparisons that was 3). We then compared haplotype sequences within *SREBF1* based on their effects on milk FA composition to determine tag SNP that could be used as genetic markers. Heritabilities of milk FA, milk production, and milk fat were calculated as ratios of an additive genetic variance to a total phenotypic variance for a particular trait with ASReml (Gilmour et al., 2009). A proportion of a total genetic variance explained by a haplotype substitution effect for significantly associated traits was

calculated as a ratio of the variance associated with the haplotype substitution effect, determined as twice the product of the haplotype frequencies multiplied by the squared estimate of the haplotype substitution effect, to the total genetic variance.

RESULTS

Analyses of the entire lactation milk FA data showed that milk atherogenic index was 2.07 (Table 1). The majority of FA in bovine milk were SFA (63.92 wt %) followed by UFA (36.08 wt %) that were primarily composed of MUFA (31.26 wt %). The PUFA concentration in milk was only 4.83 wt % with linoleic acid (18:2 *cis*-9, *cis*-12, 3.25 wt %) being the main contributor. Differences in atherogenic index (1.26–2.81), concentrations of SFA (55.51–70.29 wt %), UFA (29.71–44.49 wt %), and MUFA (25.64–38.98 wt %), and SFA/UFA ratio (1.25–2.37) among milk samples were shown by reporting 5 and 95 percentiles (Table 1). The major FA in milk was palmitic (16:0; 29.29 wt %), followed by oleic (18:1 *cis*9; 23.57 wt %), stearic (18:0; 11.93 wt %), and myristic (14:0; 10.13 wt %) acids. The C14 and C16 desaturation indices (0.07 and 0.06, respectively) of milk were low, whereas C18 desaturation index (0.66) and elongation index (0.53) were high. Summaries of milk FA composition data for the first 3 mo of lactation, and for the period between mo 4 and 10 of lactation, showed that atherogenic index, concentrations of SFA, PUFA, and SFA:UFA ratio were lower during the first 3 mo of lactation as compared with the rest of lactation period (Table 2). The opposite trend was observed for the concentrations of UFA and MUFA in milk. Concentrations of capric (10:0), lauric (12:0), myristic (14:0), and palmitic (16:0) acids, as well as conjugated linoleic acid (**CLA**; 18:2 *cis*-9, *trans*-11), were higher between mo 4 and 10 of lactation compared with the first 3 mo of lactation; the opposite trend was observed for the concentrations of butyric (4:0), caproic (6:0), stearic (18:0), and oleic (18:1 *cis*-9) acids. Except for the concentrations of caprylic (8:0) and linoleic (18:2 *cis*-9, *cis*-12) acids, the differences between all other FA concentrations in milk during the first 3 and last 7 mo of lactation were significant (data not shown).

Milk FA heritabilities (Table 2) ranged from very low to high depending on a particular FA and a period of lactation from which milk samples were analyzed. Milk samples collected between mo 4 and 10 of lactation had higher FA heritabilities as compared with milk samples collected during the first 3 mo of lactation. Heritabilities for milk production and milk fat, as well as SFA, UFA, MUFA, and PUFA calculated based on the data from the entire lactation were high, ranging from 0.23 for milk fat to 0.30 for PUFA and milk production;

Table 1. Summary of milk FA composition

Trait	Lactation period					
	mo 1 to 10		mo 1 to 3		mo 4 to 10	
	Mean (SD)	5th–95th percentile	Mean (SD)	5th–95th percentile	Mean (SD)	5th–95th percentile
Milk production, kg/d	40.17 (10.04)	24.29–57.29	42.21 (11.52)	23.61–61.02	39.28 (9.18)	24.52–54.30
Milk fat, %	3.66 (0.71)	2.50–4.80	3.73 (0.78)	2.60–5.10	3.62 (0.67)	2.50–4.70
Atherogenic index	2.07 (0.46)	1.26–2.81	1.90 (0.49)	1.13–2.73	2.15 (0.43)	1.37–2.82
SFA, wt %	63.92 (4.49)	55.51–70.29	62.90 (4.92)	54.14–70.29	64.36 (4.21)	56.40–70.29
Unsaturated FA (UFA), wt %	36.08 (4.49)	29.71–44.49	37.10 (4.92)	29.71–45.86	35.64 (4.21)	29.71–43.60
MUFA, wt %	31.26 (4.06)	25.64–38.98	32.39 (4.62)	25.66–40.64	30.80 (3.68)	25.63–37.78
PUFA, wt %	4.83 (0.93)	3.45–6.44	4.71 (0.89)	3.31–6.23	4.88 (0.94)	3.50–6.56
SFA:UFA	1.81 (0.34)	1.25–2.37	1.74 (0.36)	1.18–2.37	1.84 (0.32)	1.29–2.37
4:0, wt %	2.48 (0.75)	1.29–3.79	2.76 (0.73)	1.73–4.00	2.35 (0.73)	1.22–3.57
6:0, wt %	1.55 (0.35)	0.90–2.05	1.66 (0.36)	1.08–2.22	1.51 (0.33)	0.85–1.96
8:0, wt %	1.00 (0.23)	0.57–1.35	1.02 (0.25)	0.58–1.40	1.00 (0.23)	0.56–1.33
10:0, wt %	2.48 (0.63)	1.34–3.47	2.36 (0.67)	1.17–3.44	2.53 (0.60)	1.43–3.49
12:0, wt %	2.94 (0.73)	1.60–4.10	2.66 (0.75)	1.37–3.88	3.06 (0.69)	1.87–4.16
13:0, wt %	0.18 (0.08)	0.07–0.33	0.15 (0.07)	0.05–0.28	0.19 (0.08)	0.08–0.35
14:0, wt %	10.13 (1.61)	6.77–12.27	9.33 (1.74)	6.03–11.83	10.49 (1.41)	7.64–12.37
14:1 <i>cis</i> -9, wt %	0.79 (0.30)	0.38–1.33	0.60 (0.21)	0.30–0.98	0.87 (0.29)	0.49–1.41
15:0, wt %	1.08 (0.28)	0.71–1.63	1.00 (0.27)	0.60–1.47	1.12 (0.28)	0.80–1.68
16:0, wt %	29.29 (2.85)	24.82–34.13	28.24 (2.86)	24.05–33.56	29.75 (2.72)	25.50–34.28
16:1 <i>cis</i> -9, wt %	1.75 (0.41)	1.16–2.48	1.79 (0.40)	1.22–2.50	1.74 (0.42)	1.13–2.46
17:0, wt %	0.63 (0.09)	0.49–0.77	0.66 (0.09)	0.53–0.82	0.62 (0.08)	0.49–0.75
18:0, wt %	11.93 (2.39)	8.42–16.16	12.85 (2.42)	8.98–17.03	11.53 (2.27)	8.32–15.60
18:1 <i>cis</i> -9, wt %	23.57 (3.48)	18.81–30.13	24.52 (4.16)	18.69–32.27	23.15 (3.05)	18.83–28.95
18:2 <i>cis</i> -9, <i>cis</i> -12, wt %	3.25 (0.71)	2.22–4.49	3.21 (0.71)	2.13–4.42	3.27 (0.70)	2.26–4.51
18:2 <i>cis</i> -9, <i>trans</i> -11 CLA, ¹ wt %	0.70 (0.23)	0.43–1.17	0.66 (0.22)	0.40–1.06	0.72 (0.24)	0.44–1.21
C14 index ²	0.07 (0.02)	0.04–0.11	0.06 (0.02)	0.04–0.09	0.08 (0.02)	0.05–0.12
C16 index ³	0.06 (0.01)	0.04–0.08	0.06 (0.02)	0.04–0.09	0.06 (0.01)	0.04–0.08
C18 index ⁴	0.66 (0.05)	0.59–0.74	0.66 (0.05)	0.57–0.74	0.67 (0.05)	0.59–0.75
Elongation index ⁵	0.53 (0.05)	0.46–0.62	0.55 (0.05)	0.46–0.64	0.52 (0.05)	0.45–0.60

¹Conjugated linoleic acid.²C14 Desaturation index = 14:1/(14:0 + 14:1).³C16 Desaturation index = 16:1/(16:0 + 16:1).⁴C18 Desaturation index = 18:1/(18:0 + 18:1).⁵Elongation index = (18:0 + 18:1)/(16:0 + 16:1 + 18:0 + 18:1).

however, the heritability values were much higher when the data from only the period between mo 4 and 10 of lactation were analyzed (Table 2). The SFA/UFA ratio had a low heritability except for the period between mo 4 and 10 of lactation when the heritability was 0.14. Butyric acid (4:0) had a low heritability of 0.08 during the entire lactation that peaked during the first 3 mo of lactation and declined thereafter. In contrast, FA such as caproic (6:0) and caprylic (8:0) had heritabilities of 0.21 and 0.15, respectively, only when milk samples were collected between mo 4 and 10 of lactation. Saturated medium- and long-chain FA, such as capric (10:0), lauric (12:0), myristic (14:0), palmitic (16:0), and stearic (18:0), were highly heritable, with the highest heritability values being observed between mo 4 and 10 of lactation. Palmitic acid (16:0) had the highest heritability, 0.46, among all milk FA that was observed between mo 4 and 10 of lactation. Odd-chain SFA and MUFA—except for oleic acid (18:1 *cis*-9)—

and the desaturation and elongation indices had very low heritabilities. Among UFA, only oleic (18:1 *cis*-9) and linoleic (18:2 *cis*-9,*cis*-12) acids had high heritabilities, 0.31 and 0.27, respectively, when milk from the period between mo 4 and 10 of lactation was analyzed.

Genomic DNA sequencing in exonic and flanking intronic gene regions let us identify 5, 20, and 19 SNP in *SREBF1*, *INSIG1*, and *SCAP*, respectively (Table 3). The majority of SNP were intronic with nonsynonymous SNP *SREBF1*:g.13495T>C (*SREBF1*:p.Leu852Pro, ss526061830) and synonymous SNP *INSIG1*:g.4683C>T (ss526061846) and *SCAP*:g.51T>G (ss526061914). After genotyping animals for the discovered SNP, 3, 3, and 2 intragenic haplotypes were reconstructed for *SREBF1*, *INSIG1*, and *SCAP*, respectively (Table 4). The population frequencies for the reconstructed haplotypes exceeded 5%.

The concentration of lauric acid (12:0) in milk was significantly associated with the overall haplotype ef-

Table 2. Milk FA heritabilities

Trait	Lactation period					
	mo 1 to 10		mo 1 to 3		Mo 4 to 10	
	h ²	SE (h ²)	h ²	SE (h ²)	h ²	SE (h ²)
Milk production, kg/d	0.30	0.03	0.18	0.07	0.41	0.04
Milk fat, %	0.23	0.02	0.11	0.05	0.31	0.03
Atherogenic index	0.11	0.01	0.11	0.05	0.18	0.03
SFA, wt %	0.28	0.02	0.24	0.06	0.38	0.03
Unsaturated FA (UFA), wt %	0.28	0.02	0.24	0.06	0.38	0.03
MUFA, wt %	0.26	0.02	0.22	0.06	0.38	0.03
PUFA, wt %	0.30	0.02	0.18	0.06	0.36	0.03
SFA:UFA	0.08	0.01	0.07	0.03	0.14	0.02
4:0, wt %	0.08	0.01	0.13	0.07	0.09	0.02
6:0, wt %	0.07	0.01	0.01	0.02	0.21	0.04
8:0, wt %	0.05	0.007	0.02	0.01	0.15	0.04
10:0, wt %	0.19	0.02	0.15	0.06	0.32	0.03
12:0, wt %	0.17	0.02	0.17	0.06	0.28	0.03
13:0, wt %	0.004	0.0006	0.003	0.0008	0.005	0.001
14:0, wt %	0.18	0.02	0.22	0.06	0.28	0.03
14:1 <i>cis</i> -9, wt %	0.04	0.005	0.02	0.008	0.07	0.01
15:0, wt %	0.05	0.007	0.10	0.060	0.07	0.01
16:0, wt %	0.34	0.02	0.29	0.058	0.46	0.03
16:1 <i>cis</i> -9, wt %	0.07	0.009	0.04	0.024	0.13	0.02
17:0, wt %	0.01	0.003	0.006	0.004	0.01	0.004
18:0, wt %	0.19	0.02	0.20	0.06	0.31	0.03
18:1 <i>cis</i> -9, wt %	0.21	0.02	0.21	0.06	0.31	0.03
18:2 <i>cis</i> -9, <i>cis</i> -12, wt %	0.21	0.02	0.16	0.06	0.27	0.03
18:2 <i>cis</i> -9, <i>trans</i> -11 CLA, ¹ wt %	0.05	0.005	0.02	0.008	0.06	0.008
C14 index ²	0.0005	0.0001	0.0002	0.0001	0.0007	0.0001
C16 index ³	0.0001	0.0000	0.0001	0.0001	0.0002	0.0000
C18 index ⁴	0.002	0.0003	0.001	0.0008	0.003	0.0005
Elongation index ⁵	0.002	0.0004	0.006	0.005	0.005	0.001

¹Conjugated linoleic acid.²C14 Desaturation index = 14:1/(14:0 + 14:1).³C16 Desaturation index = 16:1/(16:0 + 16:1).⁴C18 Desaturation index = 18:1/(18:0 + 18:1).⁵Elongation index = (18:0 + 18:1)/(16:0 + 16:1 + 18:0 + 18:1).

fect of *SREBF1* (adjusted *P*-value = 0.022, Table 5) when milk FA composition data were analyzed for the period between mo 4 and 10 of lactation. Pairwise comparisons between haplotype effects for the same period of lactation showed that haplotype H1 of *SREBF1* was significantly associated with 0.098 and 0.11 wt % lower lauric acid (12:0) concentration in milk as compared with the effects of haplotypes H3 (*P*-value = 0.011) and H2 (*P*-value = 0.0075) of *SREBF1*, respectively. For the H2 and H1, H3 and H1, and H2 and H3 haplotype pairs of *SREBF1*, the haplotype substitution effect explained 1.58, 1.32, and 0.0024%, respectively, of total genetic variability with respect to lauric acid (12:0) concentration in milk measured during the period between mo 4 and 10 of lactation (Table 6).

The overall haplotype effect of *SREBF1* tested using milk FA composition data from the 10-mo lactation period was significantly associated with differences in myristic acid (14:0) concentration (adjusted *P*-value = 0.016, Table 5). Pairwise comparisons between haplo-

type effects of *SREBF1* on myristic acid (14:0) concentration in milk for the 10-mo lactation period showed that haplotype H1 of *SREBF1* was significantly associated with 0.23 wt % lower myristic acid concentration compared with the effect of haplotype H3 of *SREBF1* (*P*-value < 0.001, Table 6). Differences in myristic acid (14:0) concentration in milk were also significantly associated with the overall haplotype effect of *SREBF1* (adjusted *P*-value = 0.002, Table 5) when milk FA composition data were analyzed for the period between mo 4 and 10 of lactation. Haplotype H1 of *SREBF1* was significantly associated with 0.32 wt % lower myristic acid (14:0) concentration in milk (*P*-value < 0.0001, Table 6) as compared with the effect of haplotype H3 of *SREBF1* for the period between mo 4 and 10 of lactation. For the H2 and H1, H3 and H1, and H2 and H3 haplotype pairs of *SREBF1*, the haplotype substitution effect explained 0.21%, 3.22%, and 0.55%, respectively, of total genetic variability with respect to myristic acid (14:0) concentration in milk measured during the

Table 3. Single nucleotide polymorphisms used for the reconstruction of haplotypes in *SREBF1*, *INSIG1*, and *SCAP* genes¹

Gene	SNP	ss no.	Position, bp	Gene area	Alleles	Mutation type	AA residue change
<i>SREBF1</i>	1	ss526060904	8,729	Intron 2–3	C/T	Intronic	None
	2	ss526060908	8,837	Intron 2–3	A/G	Intronic	None
	3	ss526060911	11,598	Intron 9–10	C/G	Intronic	None
	4	ss526061830	13,495	Exon 14	T/C	Nonsynonymous	L852P
	5	ss526060915	13,801	Intron 15–16	T/C	Intronic	None
<i>INSIG1</i>	1	ss526060917	631	Intron 1–2	T/A	Intronic	None
	2	ss526060919	2,681	Intron 2–3	A/C	Intronic	None
	3	ss526061838	2,797	Intron 2–3	C/A	Intronic	None
	4	ss526061841	4,280	Intron 3–4	G/A	Intronic	None
	5	ss526061843	4,487	Intron 3–4	G/T	Intronic	None
	6	ss526061846	4,683	Exon 4	C/T	Synonymous	None
	7	ss526061848	4,772	Intron 4–5	C/G	Intronic	None
	8	ss526061851	4,796	Intron 4–5	T/C	Intronic	None
	9	ss526061853	5,157	Intron 4–5	C/T	Intronic	None
	10	ss526061856	5,454	Intron 5–6	T/C	Intronic	None
	11	ss526061859	5,501	Intron 5–6	G/A	Intronic	None
	12	ss526061861	5,727	Intron 5–6	A/G	Intronic	None
	13	ss526061864	5,790	Intron 5–6	A/G	Intronic	None
	14	ss526061866	6,757	Intron 5–6	A/G	Intronic	None
	15	ss526060943	7,413	Intron 5–6	G/A	Intronic	None
	16	ss526060945	7,580	Intron 5–6	A/C	Intronic	None
	17	ss526061872	8,900	Intron 5–6	A/G	Intronic	None
	18	ss526060949	9,952	Intron 5–6	G/A	Intronic	None
	19	ss526060950	10,782	Intron 5–6	T/C	Intronic	None
	20	ss526060952	10,825	Intron 5–6	G/C	Intronic	None
<i>SCAP</i>	1	ss526060954	21,468	Intron 11–12	G/A	Intronic	None
	2	ss526060956	19,727	Intron 9–10	G/A	Intronic	None
	3	ss526060958	12,747	Intron 2–3	G/T	Intronic	None
	4	ss526060960	12,615	Intron 2–3	A/G	Intronic	None
	5	ss526060962	11,504	Intron 2–3	G/A	Intronic	None
	6	ss526060964	8,296	Intron 2–3	G/A	Intronic	None
	7	ss526061891	8,259	Intron 2–3	T/C	Intronic	None
	8	ss526060967	5,138	Intron 2–3	A/G	Intronic	None
	9	ss526060969	4,020	Intron 2–3	G/A	Intronic	None
	10	ss526060971	4,011	Intron 2–3	A/G	Intronic	None
	11	ss526060973	3,634	Intron 2–3	A/C	Intronic	None
	12	ss526060975	2,976	Intron 2–3	C/T	Intronic	None
	13	ss526060976	2,357	Intron 2–3	A/G	Intronic	None
	14	ss526060979	2,147	Intron 2–3	G/A	Intronic	None
	15	ss526060981	2,043	Intron 2–3	A/T	Intronic	None
	16	ss526060982	1,526	Intron 2–3	T/C	Intronic	None
	17	ss526060985	747	Intron 2–3	C/G	Intronic	None
	18	ss526060987	256	5' UTR ² /Intron 1–2	T/C	Intronic	None
	19	ss526061914	51	5' UTR/Exon 1	T/G	Synonymous	None

¹*SREBF1* = sterol regulatory element binding transcription factor 1; *INSIG1* = insulin induced gene 1; *SCAP* = SREBF1 chaperone.²UTR = untranslated region.**Table 4.** Intragenic haplotypes and their frequencies

Gene ¹	Haplotype	Sequence ^{2,3}	Frequency ⁴
<i>SREBF1</i>	H1	T ₁ G ₂ C ₃ <u>T</u> ₄ C ₅	0.61
	H2	C ₁ G ₂ G ₃ C ₄ C ₅	0.19
	H3	C ₁ <u>A</u> ₂ C ₃ C ₄ <u>T</u> ₅	0.20
<i>INSIG1</i>	H1	A ₁ C ₂ C ₃ A ₄ T ₅ T ₆ G ₇ C ₈ T ₉ C ₁₀ A ₁₁ G ₁₂ G ₁₃ G ₁₄ G ₁₅ A ₁₆ G ₁₇ A ₁₈ C ₁₉ C ₂₀	0.35
	H2	A ₁ C ₂ A ₃ A ₄ T ₅ T ₆ G ₇ C ₈ T ₉ C ₁₀ A ₁₁ G ₁₂ G ₁₃ G ₁₄ A ₁₅ C ₁₆ G ₁₇ A ₁₈ T ₁₉ C ₂₀	0.28
	H3	T ₁ A ₂ C ₃ G ₄ G ₅ C ₆ C ₇ T ₈ C ₉ T ₁₀ G ₁₁ A ₁₂ A ₁₃ A ₁₄ G ₁₅ A ₁₆ A ₁₇ G ₁₈ T ₁₉ G ₂₀	0.33
<i>SCAP</i>	H1	G ₁ G ₂ G ₃ A ₄ G ₅ G ₆ T ₇ A ₈ G ₉ A ₁₀ A ₁₁ C ₁₂ A ₁₃ G ₁₄ A ₁₅ T ₁₆ C ₁₇ T ₁₈ G ₁₉	0.11
	H2	G ₁ G ₂ G ₃ A ₄ G ₅ G ₆ T ₇ A ₈ G ₉ A ₁₀ A ₁₁ C ₁₂ A ₁₃ G ₁₄ A ₁₅ T ₁₆ C ₁₇ T ₁₈ T ₁₉	0.75

¹*SREBF1* = sterol regulatory element binding transcription factor 1; *INSIG1* = insulin induced gene 1; *SCAP* = SREBF1 chaperone.²Subscripts are SNP numbers in a particular haplotype for a particular gene.³The tag SNP in the haplotypes of interest are underlined.⁴Only haplotypes with the population frequencies $\geq 5\%$ were considered in the analysis

Table 5. Results of F-test for the overall haplotype effect of sterol regulatory element binding transcription factor 1 (*SREBF1*) on milk FA composition

Trait	Lactation period								
	mo 1 to 10			mo 1 to 3			mo 4 to 10		
	F-value	P-value	Adjusted P-value	F-value	P-value	Adjusted P-value	F-value	P-value	Adjusted P-value
Milk production	3.09	0.047	0.14	0.86	0.42	0.63	5.05	0.007	0.014*
Milk fat	0.99	0.38	0.57	0.03	0.97	0.97	1.23	0.29	0.78
Atherogenic index	1.05	0.35	0.35	0.43	0.65	0.88	2.12	0.12	0.22
SFA	0.58	0.56	0.56	0.12	0.89	0.89	1.22	0.30	0.30
Unsaturated FA (UFA)	0.58	0.56	0.56	0.12	0.89	0.89	1.22	0.30	0.30
MUFA	0.62	0.54	0.54	0.17	0.84	0.93	1.33	0.27	0.27
PUFA	1.00	0.37	0.39	0.23	0.79	0.79	1.04	0.36	0.54
SFA:UFA	0.59	0.56	0.56	0.11	0.89	0.90	1.14	0.32	0.32
4:0	0.56	0.57	0.57	0.26	0.77	0.77	0.36	0.70	0.70
6:0	1.21	0.30	0.44	0.53	0.59	0.89	1.40	0.25	0.38
8:0	1.81	0.17	0.47	0.47	0.63	0.74	2.59	0.077	0.23
10:0	2.32	0.10	0.30	1.04	0.36	0.69	3.85	0.023	0.069
12:0	2.39	0.09	0.27	0.63	0.53	0.70	4.54	0.011	0.022*
13:0	0.53	0.59	0.59	0.51	0.60	0.75	1.28	0.28	0.73
14:0	4.97	0.008	0.016*	1.36	0.26	0.78	8.13	<0.001	0.002*
14:1 <i>cis</i> -9	3.15	0.05	0.075	4.10	0.018	0.054	2.51	0.084	0.13
15:0	0.68	0.51	0.77	0.81	0.45	0.72	0.85	0.43	0.72
16:0	0.51	0.60	0.60	0.32	0.72	0.72	0.51	0.60	0.60
16:1 <i>cis</i> -9	1.26	0.29	0.29	0.17	0.84	0.84	2.22	0.11	0.20
17:0	0.49	0.61	0.92	0.69	0.50	0.75	0.17	0.85	0.95
18:0	1.17	0.31	0.88	0.57	0.57	0.73	1.17	0.31	0.56
18:1 <i>cis</i> -9	0.94	0.39	0.59	0.63	0.53	0.80	2.28	0.11	0.33
18:2 <i>cis</i> -9, <i>cis</i> -12	1.63	0.20	0.51	0.50	0.61	0.67	1.69	0.19	0.57
18:2 <i>cis</i> -9, <i>trans</i> -11 CLA ¹	0.40	0.67	0.67	0.55	0.58	0.58	0.28	0.75	0.75
Index 14 ²	3.28	0.039	0.059	2.26	0.11	0.17	3.46	0.033	0.05
Index 16 ³	0.79	0.46	0.46	0.39	0.67	0.67	1.81	0.17	0.17
Index 18 ⁴	0.55	0.58	0.58	0.09	0.92	0.96	0.58	0.56	0.56
Elongation index ⁵	0.24	0.78	0.80	0.64	0.53	0.64	0.34	0.71	0.87

¹Conjugated linoleic acid.²C14 Desaturation index = 14:1/(14:0 + 14:1).³C16 Desaturation index = 16:1/(16:0 + 16:1).⁴C18 Desaturation index = 18:1/(18:0 + 18:1).⁵Elongation index = (18:0 + 18:1)/(16:0 + 16:1 + 18:0 + 18:1).*Significance of haplotype effects was declared if adjusted *P*-value was <0.05.

entire lactation period (Table 6). The same analysis performed with respect to myristic acid (14:0) concentration in milk measured during the period between mo 4 and 10 of lactation showed that the percentages were 0.82, 4.53, and 0.45%, respectively.

Milk production was significantly associated with the overall haplotype effect of *SREBF1* (adjusted *P*-value = 0.014, Table 5) when data collected between mo 4 and 10 of lactation period were analyzed. Pairwise comparisons between haplotype effects on milk production revealed that haplotype H1 of *SREBF1* was significantly associated with 1.63 kg/d lower milk production compared with the effect of haplotype H3 of *SREBF1* (*P*-value < 0.001, Table 6) when analyzing the data for the same lactation period. For the H2 and H1, H3 and H1, and H2 and H3 haplotype pairs of *SREBF1*, the haplotype substitution effect explained 0.13, 3.67, and 0.74%, respectively, of total genetic variability with

respect to milk production measured during the period between mo 4 and 10 of lactation (Table 6).

The comparisons between *SREBF1* haplotype sequences (Table 4) revealed a tag SNP—*SREBF1*:g.13495T>C (*SREBF1*:p.Leu852Pro, ss526061830)—that distinguishes the most desirable haplotype H1 of *SREBF1* from remaining *SREBF1* haplotypes when selecting for milk healthfulness. Another 2 tag SNP—*SREBF1*:g.8837A>G (ss526060908) and *SREBF1*:g.13801T>C (ss526060915)—were identified (Table 4), and either of those SNP can be used to distinguish haplotype H3 of *SREBF1* from remaining *SREBF1* haplotypes when selecting for high milk yield. Protein sequence alignment of SREBP1 from human, gorilla, pig, rat, mouse, and cow showed that Pro 852 on the cow protein sequence was highly conserved among mammalian species (Figure 1). No significant associations were detected for the overall haplotype effects of

Table 6. Pairwise comparisons of sterol regulatory element binding transcription factor 1 (*SREBF1*) haplotype effects on milk production and FA composition¹

Trait	Lactation period	H2–H1	R ² _{genetic} , ² %	H3–H1	R ² _{genetic} , %	H2–H3	R ² _{genetic} , %
14:0	mo 1 to 10	0.060 (0.073)	0.21	0.23 (0.072)	3.22	−0.17 (0.089)	0.55
Milk production	mo 4 to 10	0.21 (0.52)	0.13	<0.001 (0.51)	3.67	0.031 (0.63)	0.74
		0.27 (0.043)		<0.001 (0.042)		0.020 (0.053)	
12:0	mo 4 to 10	0.11 (0.043)	1.58	0.098 (0.042)	1.32	0.0075 (0.053)	0.0024
14:0	mo 4 to 10	0.0075 (0.082)	0.82	0.011 (0.080)	4.53	0.56 (0.099)	0.45
		0.14 (0.044)		<0.0001		0.037	

¹H_a–H_b = a pair of haplotypes for which the haplotype effects on milk FA composition were compared. The first value for each trait is an estimate of the haplotype substitution effect expressed in weight percentage for all individual FA and FA groups and in kilograms for milk production, the value in parentheses is the SE of an estimate of the haplotype substitution effect expressed in the same units as the estimate, and the third value is a *P*-value.

²R²_{genetic} = percentage of genetic variance explained by a haplotype substitution effect.

*Significance was declared if *P*-value was ≤0.0167.

INSIG1 and *SCAP* with any of the traits studied when the cut off *P*-value of 0.017 was considered after applying the Bonferroni adjustment to account for testing 3 genes at the same time (Supplementary Tables S3 and S4; <http://www.journalofdairyscience.org/>).

DISCUSSION

To test associations between milk FA and genetic polymorphisms in the SREBP pathway, statistical analyses were performed on milk FA composition data from the 10-mo lactation period (305-d lactation) and from the first 3 and the last 7 mo of the 10-mo lactation period. The rationale for analyzing FA acid composition data separately for different parts of lactation is based on the fact that heritabilities of milk FA are higher during mid and late lactation compared with the first 3 mo of lactation (Bastin et al., 2011; Gross et al., 2011). Existence of higher FA heritabilities in milk samples collected after the first 3 mo of lactation is also supported by our data (Table 2). The greater

contribution of de novo lipogenesis and smaller contribution of lipolysis from adipose tissues toward milk lipid biosynthesis in dairy cattle are the main reasons for higher milk FA heritabilities during late two-thirds of lactation.

Mean values for the major milk FA in our study were similar to those reported by others (Jensen, 2002; Mansson, 2008; Stoop et al., 2008). Differences in atherogenic index and SFA, UFA, MUFA, and PUFA concentrations—as well as concentrations of myristic (14:0) and palmitic (16:0) acids in milk determined by calculating 5th and 95th percentiles—were large, indicating a great degree of variability in milk FA composition and a possibility of obtaining milk samples with low atherogenic index and low concentrations of SFA and palmitic (16:0) and myristic (14:0) acids and high concentrations of UFA, MUFA, and PUFA. Comparison of milk FA composition data from different parts of lactation showed that milk produced during the first 3 mo of lactation had a healthier FA profile and smaller atherogenic index compared with the milk produced

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Human      GYLQLLNSCSDAAGAPAYSFSISSSMATTTGVDPVAKWWASLTAVVIHWLRRDEEAAERL 897
Gorilla    GYLQLLNSCSDAAGAPACSFSSISSSMATTTGVDPVAKWWASLTAVVIQPCID----- 890
Cow        GYLQLLNSCSDVAGAPTCSFSISSSMATTPGTDPAKWWASLTAVVTHWLRDEEAAERL 896
Pig        GFLQLLNSCCDTAGAPACSFSSIASSTAATAGTDPVAKWWASLTAVVTHWLGRRDEEAAERL 897
Rat        GYLQLLNSCSDAVGAPACSFSSVSSSMATTTGTDPVAKWWASLTAVVIHWLRRDEEAAERL 884
Mouse      GYLQLLNSCSDAAGAPACSFSSVSSSMATTTGPDPAKWWASLTAVVIHWLRRDEEAAERL 884

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Figure 1. Multiple alignment of sterol regulatory element binding transcription factor 1 protein sequences from mammalian species. The position of Pro852Leu mutation is highlighted in bold. Numbers on the right are relative positions of AA. The accession numbers for protein sequences are NP_004167.3 (human), ENSGGOP00000002678 (gorilla), NP_001106773.1 (cow), NP_999322.1 (pig), P56720.3 (rat), and NP_035610.1 (mouse).

between mo 4 and 10 of lactation. Low values for C14 and C16 desaturation indices and high values for C18 desaturation index and elongation index indicate that the major pathway for the conversion of myristic (14:0) and palmitic (16:0) acids to oleic acid (18:1 *cis*-9) includes elongation of myristic (14:0) and palmitic (16:0) acids to stearic acid (18:0) with subsequent desaturation to oleic acid (18:1 *cis*-9). Synthesis of oleic acid (18:1 *cis*-9) through the elongation of myristoleic (14:1) and palmitoleic (16:1) acids is not a major pathway for oleic acid (18:1 *cis*-9) biosynthesis and this information has to be taken into account when developing strategies to change milk FA composition.

Overall, milk FA heritabilities observed in our study were very similar to those reported earlier (Schennink et al., 2007; Soyeurt et al., 2007; Stoop et al., 2008; Bastin et al., 2011; Bouwman et al., 2011). Our data also confirmed that milk FA heritabilities are changing throughout the lactation period and are much higher after the first 3 mo of lactation, as shown previously by Bastin et al. (2011). Heritabilities of odd chain SFA, MUFA—except for oleic acid (18:1 *cis*-9)—butyric acid (4:0), CLA (18:2 *cis*-9,*trans*-11), and the desaturation and elongation indices were much lower in our study compared with data reported earlier. Nevertheless, the heritabilities for major milk FA were high, supporting the idea of improving milk healthfulness through genetics.

In our study, haplotype H1 of *SREBF1* was associated with low concentration of myristic acid (14:0) in milk when data from the entire 10-mo lactation and from the period between mo 4 and 10 of lactation were analyzed. The size of H1 haplotype effect on myristic acid (14:0) concentration in milk, however, was larger between mo 4 and 10 of lactation as compared with H1 haplotype effect during the entire 10-mo lactation period. The difference in H1 haplotype effect on myristic acid (14:0) concentration during different parts of lactation can be explained by the fact that myristic acid (14:0) is primarily synthesized by de novo lipogenesis in mammary gland and its concentration in bovine adipose tissue is minimal (Pothoven et al., 1974). This explains why no significant associations were noted between the overall haplotype effect and myristic acid (14:0) concentration in milk during the first 3 mo of lactation when lipolysis in adipose tissue and not de novo lipogenesis in mammary gland is the primary source of milk FA (Palmquist et al., 1993).

Haplotype H1 of *SREBF1* was also associated with low concentration of lauric acid (12:0) during the period between mo 4 and 10 of lactation. Similar to myristic acid (14:0), lauric acid (12:0) concentration in milk was not significantly associated with the overall haplotype effect during the first 3 mo of lactation because lauric

acid (12:0) is primarily synthesized by de novo lipogenesis in the mammary gland that is fully functional only after the first 3 mo of lactation (Pothoven et al., 1974; Palmquist et al., 1993). Also noted was an association of haplotype H1 of *SREBF1* with lower milk production compared with the effect of haplotype H3 of *SREBF1* that makes the selection for milk healthfulness undesirable with respect to milk production. Otherwise, haplotype H1 of *SREBF1* was the most desirable to select for milk healthfulness. A potential benefit of selecting for haplotype H1 of *SREBF1* to improve milk healthfulness was also supported by the highest proportion of total genetic variance that was explained by haplotype H1 over haplotype H3 substitution effect as compared with other *SREBF1* haplotype substitution effects (Table 6).

The tag SNP—*SREBF1*:g.13495T>C (*SREBF1*:p.Leu852Pro, ss526061830)—for haplotype H1 of *SREBF1* might be responsible for the haplotype effects on milk FA composition and milk production. The SREBP1 AA residue at position 852 was not a part of any known homology domains, but the residue was within the SREBP1 C-terminal regulatory domain responsible for sterol-regulated proteolytic cleavage of SREBP1 (Brown and Goldstein, 1997). Multiple alignment of SREBP1 protein sequences from human, gorilla, cow, pig, rat, and mouse (Figure 1) revealed that Pro 852 of a cow SREBP1 sequence was highly conserved among mammalian species, implying its importance for SREBP1 protein function. In our animal population, however, the presence of Leu 852 of SREBP1 was more prevalent (Table 4), possibly as a result of animal selection in our study herds.

A QTL for myristic acid (14:0) concentration in milk was located on BTA19 at 68 cM (confidence interval 51–77 cM), and *FASN* located on BTA19 at 72 cM was identified as a positional candidate gene for the QTL (Morris et al., 2007). A different QTL for myristic acid (14:0) concentration in milk was located on BTA19 at 59 cM (confidence interval 38–83 cM), but no positional genes were identified for the QTL (Stoop et al., 2009). It was speculated, however, that the QTL on BTA19 at 59 cM might not only be associated with the effects of *FASN*, but also can be associated with the effects of signal transducer and activator of transcription 5A (*STAT5A*; 56 cM; Brym et al., 2004) or growth hormone (69 cM; Viitala et al., 2006) that were previously shown to be associated with milk fat yield. In a subsequent study, the QTL for myristic acid (14:0) concentration in milk on BTA19 at 59 cM was tested for associations with SNP in *FASN* and *STAT5A*, those SNP were not able to explain the majority of phenotypic effects seen for the QTL that was still significant after making the adjustments for the effects of *FASN* and *STAT5A* (Schennink et al., 2009). In a different

genome-wide association study, a QTL for myristic acid concentration in milk was positioned on BTA19 at 46 to 87 cM (32.7–61.8 Mbp; Bouwman et al., 2011). It was suggested that polymorphisms in *FASN* were not solely responsible for the observed QTL and other genes located in the QTL region, such as *SREBF1*, *STAT5A*, growth hormone, and ATP citrate lyase, might be contributing to the observed phenotype. The QTL for myristic acid (14:0) concentration in milk reported in earlier studies might be explained in part by polymorphisms in *SREBF1* located on BTA19 at 50 cM that were determined in our study.

Polymorphisms in *SREBF1* were tested earlier for association with milk FA composition (Rincon et al., 2012). Only milk fat percentage was significantly associated with nonsynonymous SNP *SREBF1*:g.13495T>C (rs41912290) when data from a herd with milk fat depression were analyzed. The SNP *SREBF1*:g.13495T>C (ss526061830) was also discovered by DNA sequencing in our study and was identified to be a tag SNP for haplotype H1 of *SREBF1*. The results of our study for haplotype H1 of *SREBF1* that contained SNP *SREBF1*:g.13495T>C, however, are completely different from the results reported by Rincon et al. (2012). The association of *SREBF1* genetic polymorphism characterized by the presence of the 84-bp indel (insertion-deletion) in intron 5 was studied in Italian Brown cattle with respect to variations in milk FA composition, but no significant associations were found (Conte et al., 2010). In our study, *INSIG1* and *SCAP* genetic polymorphisms were not significantly associated with milk FA composition, in agreement with earlier reported data by Rincon et al. (2012).

CONCLUSIONS

In summary, the results of our study showed that haplotype H1 of *SREBF1* was the most desirable to select for animals producing healthier milk because that haplotype was significantly associated with a low concentration of myristic acid (14:0) during the entire 10-mo lactation period. Haplotype H1 of *SREBF1* was also significantly associated with low concentrations of lauric (12:0) and myristic (14:0) acids between mo 4 and 10 of lactation. However, haplotype H1 of *SREBF1* was also significantly associated with low milk production during the same period. Therefore, genetic selection for milk healthfulness using haplotype H1 of *SREBF1* might result in selection for decreased milk production. A tag SNP for haplotype H1 of *SREBF1* was identified to be *SREBF1*:g.13495T>C (*SREBF1*:p.Leu852Pro, ss526061830) and was located in SREBP1 regulatory C-terminal domain and was highly conserved among multiple mammalian species. The muta-

tion might be responsible for *SREBF1* H1 haplotype effects on milk FA composition and milk production. Genetic polymorphisms in *INSIG1* and *SCAP* did not show any significant associations with milk FA composition. To the best of our knowledge, our study was one of the first to use milk FA composition data that were collected monthly for each cow throughout a 305-d lactation period to test for associations with genetic polymorphisms. Moreover, in our statistical analysis, we took into account the correlation that exists between milk samples collected from the same cow to enable the computation of correct error terms in the analysis. In conclusion, information about genetic polymorphisms in *SREBF1* can be used to develop genetic markers to select for animals producing healthier milk.

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