A quantitative trait locus on *Bos taurus* autosome 17 explains a large proportion of the genetic variation in de novo synthesized milk fatty acids

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ABSTRACT

A genomic region associated with milk fatty acid (FA) composition has been detected on *Bos taurus* autosome (BTA)17 based on 50,000 (50K) single nucleotide polymorphism (SNP) genotypes. The aim of our study was to fine-map BTA17 with imputed 777,000 (777K) SNP genotypes to identify candidate genes associated with milk FA composition. Phenotypes consisted of gas chromatography measurements of 14 FA based on winter and summer milk samples. Phenotypes and genotypes were available on 1,640 animals in winter milk, and on 1,581 animals in summer milk samples. Single-SNP analyses showed that several SNP in a region located between 29.0 and 34.0 Mbp were in strong association with C6:0, C8:0, and C10:0. This region was further characterized based on haplotypes. In summer milk samples, for example, these haplotypes explained almost 10% of the genetic variance in C6:0, 9% in C8:0, 3.5% in C10:0, 1.8% in C12:0, and 0.9% in C14:0. Two groups of haplotypes with distinct predicted effects could be defined, suggesting the presence of one causal variant. Predicted haplotype effects tended to increase from C6:0 to C14:0; however, the proportion of genetic variance explained by the haplotypes tended to decrease from C6:0 to C14:0. This is an indication that the quantitative trait locus (QTL) region is involved either in the elongation process or in early termination of de novo synthesized FA. Although many genes are present in this QTL region, most of these genes on BTA17 have not been characterized yet. The strongest association was found close to the progesterone receptor membrane component 2 (*PGRMC2*) gene, which has not yet been associated with milk FA composition. Therefore, no clear candidate gene associated with milk FA composition could be identified for this QTL.

Key words: milk fatty acid composition, dairy cattle, candidate genes, high-density genotyping

INTRODUCTION

Bovine milk fat is composed of more than 400 different FA, many of which are still unidentified (Jensen, 2002). Fatty acids may differ in the number of carbons and this difference can be related to the origin of the FA. Most short-chain FA have fewer than 12 carbons that are mainly elongated from acetate by de novo synthesis in the mammary gland of a cow (e.g., Palmquist, 2006). Medium-chain FA are those with 14 and 16 carbons and, although C14:0 mainly originates from de novo synthesis, C16:0 originates from 2 sources: approximately 50% from de novo synthesis and 50% from the diet of a cow. Most long-chain FA have 18 or more carbons and mainly originate from the cow's diet or from mobilization of body fat (e.g., Chilliard et al., 2000). In addition to differences in the number of carbons, FA may also differ in their degree of saturation. On average, more than 70% of the identified FA in milk consist are saturated FA and the remainder are unsaturated FA.

Variation in the content of several FA in milk is affected by genetic factors. Stoop et al. (2008) reported that individual milk FA have heritability estimates that range from 0.22 to 0.71. Some well-characterized genes are recognized as having large effects on milk fat and FA composition, such as acyl-CoA:diacylglycerol acyltransferase1 (*DGAT1*), located on BTA14, and stearoyl-CoA desaturase1 (*SCD1*), located on BTA26 (e.g., Schennink et al., 2007, 2008). In addition, several regions of the bovine genome have been identified as having effects on milk fat and FA composition but have not yet been characterized (e.g., Bouwman et al., 2012). By fine-mapping these regions, it is possible to identify candidate genes (Ishii et al., 2013) associated with milk FA composition. Further insights into the biosynthesis of milk fat and FA are relevant if the aim is to change milk FA composition by means of breeding (Boichard and Brochard, 2012) or feeding strategies.

Fine-mapping allows refinement of genomic regions by testing a large number of SNP that are likely associated with a QTL (Hinds et al., 2005). Recently, a genomic region associated with short-chain FA in

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milk was detected on BTA17 (Bouwman et al., 2012). However, no candidate gene or causal variant has been identified so far. The aim of our study was to fine-map BTA17 with imputed 777,000 (**777K**) SNP genotypes to identify candidate genes associated with milk FA composition.

MATERIALS AND METHODS

This study is part of the Dutch Milk Genomics Initiative that aims to explore possibilities to modify milk FA composition through breeding. Bouwman et al. (2012) performed a genome-wide association study (**GWAS**) using 50,000 (**50K**) SNP genotypes based on milk FA composition of winter and summer milk samples. In the present study, we reanalyzed the same phenotypes and fine-mapped BTA17 using imputed 777K SNP genotypes.

Animals and Phenotypes

Morning milk samples (500 mL/cow) were retrieved from 2,001 first-lactation Holstein-Friesian cows from 398 herds throughout the Netherlands. At least 3 cows per herd were sampled in 2 distinct seasons: February to March 2005 (winter samples) and May to June 2005 (summer samples). The milk samples were taken from the same cows during the same lactation. Some cows sampled in winter were no longer lactating when summer milk samples were taken. Additional cows were sampled from the same herds to guarantee milk samples from at least 3 cows per herd. A total of 1,905 cows had phenotypic records in winter, with each cow lactating between 63 and 282 d (see Stoop et al., 2008). A total of 1,795 cows had phenotypic records in the summer, with each cow lactating between 97 and 335 d (see Duchemin et al., 2013). About 50% of the cows in our experiment had access to pasture in summer (3.5 to 24 h/d), whereas all cows were kept indoors and fed silage in winter. Further details about the experimental design can be found in Stoop et al. (2008).

Milk FA composition was measured by GC at the COKZ laboratory (Qlip, Leudsen, the Netherlands). Milk fat was extracted from the milk samples, and FAME were prepared from fat fractions, as described by Schennink et al. (2007). The FA were identified and quantified by comparing the methyl ester chromatograms of the milk fat samples with the chromatograms of pure FAME standards (Stoop et al., 2008). The FA included in this study were measured as weight proportion of total fat (% wt/wt) and are described in Table 1. In addition, an indicator of de novo synthesized milk FA was created by combining C6:0 through C14:0 individual FA in the index designated “C6:0–C14:0.”

Genotypes and Imputation

A blood sample from each cow and semen from each bull were used to extract DNA. The DNA of 55 sires and 1,813 daughters belonging to our experimental population was genotyped with a 50K SNP chip. This chip was designed by CRV (Arnhem, Netherlands), and was used to genotype the animals using the Infinium assay (Illumina Inc., San Diego, CA).

A reference population of 1,333 animals belonging to CRV and including the 55 sires with offspring in our data set was additionally genotyped with a 777K SNP chip (Illumina Inc., San Diego, CA). This information on the reference population was used to impute the genotypes of our experimental population from 50K to 777K SNP. This imputation was done using Beagle version 3.2.2 (Browning and Browning, 2009) and resulted in a total of 1,736 animals being imputed to 777K SNP. Of these 1,736 animals, 12 animals were excluded because of pedigree inconsistencies and 3 animals were subsequently excluded because their herds no longer met the requirement of a minimum of 3 animals sampled per herd. Consequently, 1,721 animals with imputed 777K SNP genotypes were available for this study. Imputation of BTA17 increased the number of SNP genotypes from 1,562 (i.e., 50K) to 22,240 (i.e., 777K). The positions of the imputed SNP were based on the bovine genome assembly UMD 3.1 (Zimin et al., 2009).

Fine-Mapping of BTA17

The fine-mapping of BTA17 was performed separately for winter and summer milk samples by using imputed 777K SNP genotypes and the 14 FA described in Table 1. For each season, animals were included in the analyses if both phenotypic and genotypic data were available. Therefore, 1,640 animals were available for winter milk, and 1,581 animals were available for summer milk samples.

Single SNP analyses were performed using the following animal model:

$$y_{ijklmno} = \mu + b_1 \times \text{dim}_i + b_2 \times e^{-0.05 \times \text{dim}_i} + b_3 \times \text{afc}_j \\ + b_4 \times \text{afc}_j^2 + \text{season}_k + \text{scode}_l \\ + \text{SNP}_m + \text{herd}_n + a_o + e_{ijklmno}, \quad [1]$$

where $y_{ijklmno}$ is the dependent variable; μ is the overall mean; b_1 and b_2 are the regression coefficients related to dim_i ; dim_i is the covariate describing the effect of days in milk, modeled with a Wilmink curve (Wilmink, 1987); b_3 and b_4 are the regression coefficients related to afc_j ; afc_j is the covariate describing the effect of age at

first calving; $season_k$ is the fixed effect of calving season (June–August 2004, September–November 2004, or December 2004–February 2005); $scode_l$ is the fixed effect accounting for differences in genetic level between groups of proven bull daughters and young bull daughters; SNP_m is the fixed effect of SNP genotype; $herd_n$ is the random effect of herd, and is assumed to be distributed as $N \sim (0, \mathbf{I}\sigma_{herd}^2)$, for which $\mathbf{I}$ is the identity matrix, and σ_{herd}^2 is the herd variance; a_o is the random additive genetic effect of animal, and is assumed to be distributed as $N \sim (0, \mathbf{A}\sigma_a^2)$, where $\mathbf{A}$ is the additive genetic relationships matrix which consisted of 12,548 animals, and σ_a^2 is the additive genetic variance; and $e_{ijklmno}$ is the random residual effect, and is assumed to be distributed as $N \sim (0, \mathbf{I}\sigma_e^2)$, for which $\mathbf{I}$ is the identity matrix, and σ_e^2 is the residual variance.

Additive genetic and herd variances were estimated without the inclusion of SNP information, and the resulting estimates were fixed within model [1].

Heritability estimates were calculated from univariate analyses based on model [1] without the inclusion of SNP effects as follows: $h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$. Analyses were performed separately for winter and summer milk samples. All statistical analyses were performed using ASReml 3.0 (Gilmour et al., 2009).

Construction of Haplotypes

Haplotypes were constructed to further characterize a genomic region on BTA17, and these were constructed separately for winter and summer milk samples. This construction started with the identification of promising SNP by single-SNP analyses using model [1]. The SNP with the highest significance was defined as **QTagSNP1**. Subsequently, we corrected for the effect of QTagSNP1 by including QTagSNP1 as a fixed effect in model [1]. This correction allowed us to run a second round of single-SNP analyses and retrieve remaining significant SNP. After this second round of analyses, if another SNP still was significant, it was defined as **QTagSNP2**. In these analyses, a SNP was considered to be significant if $-\log_{10}(P\text{-value}) \geq 3$. Next, we corrected for the effects of QTagSNP2 in the model already extended with QTagSNP1 by further including QTagSNP2 as a fixed effect. This methodology was repeated until no additional significant SNP were retrieved. Linkage disequilibrium (**LD**) was estimated as the squared correlation (r^2) between all the identified QTagSNP using PLINK version 1.07 (Purcell et al., 2007). After the identification of QTagSNP, haplotypes were constructed based on the identified QTagSNP.

Effects of haplotypes were estimated with the following animal model:

$$y_{ijklnpqr} = \mu + b_1 \times \text{dim}_i + b_2 \times e^{-0.05 \times \text{dim}_i} + b_3 \times \text{afc}_j + b_4 \times \text{afc}_j^2 + \text{season}_k + \text{scode}_l + \text{haplo1}_p + \text{haplo2}_q + \text{herd}_n + a_r^* + e_{ijklnpqr}, \quad [2]$$

where variables are as previously described for model [1]; haplo1_p is the random effect of the first haplotype; haplo2_q is the random effect of the second haplotype, and they are both assumed to be distributed as $N \sim (0, \mathbf{I}\sigma_{haplo}^2)$, for which $\mathbf{I}$ is the identity matrix and σ_{haplo}^2 is the haplotype variance. The first and second haplotypes were jointly used to estimate one haplotype variance (σ_{haplo}^2) and one effect for each haplotype. This was achieved by combining the design matrices of both haplotypes in ASReml; σ_r^* is the random additive genetic effect of animal estimated without the inclusion of haplotypes and is assumed to be distributed as $\sim N(0, \mathbf{A}\sigma_{a^*}^2)$, for which $\mathbf{A}$ is the additive genetic relationships matrix, which consisted of 12,548 animals, and $\sigma_{a^*}^2$ is the additive genetic variance that remains after accounting for haplotype effects. The total additive genetic variance was defined as $\sigma_a^2 = \sigma_{a^*}^2 + \sigma_{haplo}^2$. The fraction of genetic variance explained by haplotypes was defined as $\sigma_{haplo}^2 / \sigma_a^2$.

Additionally, we tested whether predicted haplotype effects differed from each other. Significance levels of the differences between predicted effects of haplotypes were assessed using Student's *t*-tests, as implemented in ASReml. The predicted effect of a haplotype was considered significantly different from another haplotype if $P\text{-value} \leq 0.05$.

RESULTS

Phenotypic Means and Heritability Estimates

Phenotypic means and heritability estimates for milk FA composition in winter and summer milk samples are shown in Table 1. Winter milk had higher contents of short-chain FA than summer milk samples (14.2 vs. 13.7%), higher contents of medium-chain FA (44.2 vs. 40.4%), and lower contents of long-chain FA, such as C18:0 (8.7 vs. 9.9%) and *cis*-9 C18:1 (18.2 vs. 20.5%). Phenotypic variance was higher in summer compared with winter milk samples, but genetic variances were similar in both seasons. A detailed discussion on differences between winter and summer milk samples can be found in our previous study (Duchemin et al., 2013).

Table 1. Phenotypic means (SD), and heritability estimates (h^2)¹ for individual FA based on 1,640 winter milk samples and 1,581 summer milk samples

Individual FA (% wt/wt)	Winter		Summer	
	Mean (SD)	h^2	Mean (SD)	h^2
Saturated FA				
C4:0	3.51 (0.27)	0.47	3.52 (0.35)	0.41
C6:0	2.23 (0.16)	0.46	2.17 (0.21)	0.39
C8:0	1.36 (0.14)	0.59	1.32 (0.17)	0.35
C10:0	3.02 (0.43)	0.73	2.87 (0.45)	0.48
C12:0	4.12 (0.70)	0.62	3.79 (0.72)	0.48
C14:0	11.62 (0.92)	0.62	11.16 (1.05)	0.54
C16:0	32.62 (2.84)	0.47	29.20 (3.49)	0.40
C18:0	8.71 (1.39)	0.28	9.86 (1.77)	0.19
Unsaturated FA				
C10:1 ²	0.37 (0.07)	0.35	0.35 (0.07)	0.50
C12:1 ²	0.12 (0.03)	0.38	0.11 (0.03)	0.47
<i>cis</i> -9 C14:1 ³	1.36 (0.25)	0.35	1.38 (0.28)	0.43
<i>cis</i> -9 C16:1	1.45 (0.32)	0.44	1.40 (0.30)	0.38
<i>cis</i> -9 C18:1 ⁴	18.18 (2.05)	0.22	20.53 (2.76)	0.35
<i>cis</i> -9, <i>trans</i> -11 C18:2 (CLA)	0.39 (0.11)	0.55	0.56 (0.27)	0.27

¹ $h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2)$, where h^2 is the heritability estimate, σ_a^2 is the additive genetic variance, and σ_e^2 is the residual variance; SE between 0.01 and 0.12 for winter samples, and between 0.02 and 0.08 for summer samples.

²For C10:1 and C12:1, the *cis* double bond could not be ascertained at the carbon 9 position.

³*cis*-9 C14:1 represents the sum of *cis*-9 C14:1 and *iso* C15 due to co-elution associated with the GC extraction method.

⁴*cis*-9 C18:1 represents the sum of *cis*-9 C18:1 and *trans*-12 C18:1 due to co-elution associated with the GC extraction method.

Fine-Mapping of BTA17

Results of the fine-mapping of BTA17 for winter and summer milk samples are shown in Supplemental Figure S1 (<http://dx.doi.org/10.3168/jds.2014-8178>). For both seasons, we analyzed associations between 22,240 imputed SNP and each of the 14 FA. In a region between 29.0 and 34.0 Mbp, multiple SNP showed highly significant associations with C6:0, C8:0, and C10:0. Moreover, multiple SNP showed associations both in winter and summer milk samples (Supplemental Figure S1). Previously, Bouwman et al. (2012) identified associations of multiple regions on BTA17 with C6:0, C8:0, C10:0, C14:1, and C16:1. Detailed analyses in the current study focused on the region between 29.0 and 34.0 Mbp because the strongest and most consistent associations were found in this region across winter and summer milk samples.

Figure 1A illustrates the strongest associations found with the imputed 777K SNP genotypes for C8:0 in summer milk samples. Additionally in Figure 1A, these associations were overlaid with the associations found by Bouwman et al. (2012) using 50K SNP genotypes, which were mainly the same data as used in the current study. Within the marked region (Figure 1A), 10 significant SNP were found with the 50K SNP, whereas 83 significant SNP were found with the imputed 777K SNP. The most significant SNP identified based on the imputed 777K SNP [$-\log_{10}(P\text{-value}) = 7.93$] was not

present on the 50K SNP array. The most significant SNP identified based on the 50K SNP genotypes was less significant [$-\log_{10}(P\text{-value}) = 6.21$; Bouwman et al., 2012] than the most significant SNP identified in the present study. The location of the QTL could be refined to the genomic region located between 29.0 and 34.0 Mbp on BTA17 (Figure 1A). Figure 1B shows the results of the associations for 5 FA in summer milk samples for this region.

Construction of Haplotypes

The construction of haplotypes was based on the identified QTagSNP in the fine-mapping of BTA17. These SNP, QTagSNP1 and QTagSNP2, were different for winter and summer milk samples. For winter milk samples, QTagSNP1 was BovineHD1700008470 (rs109426433) located at 29.92 Mbp, and with minor allele frequency (MAF) of 0.47. QTagSNP1 was associated with C6:0 [$-\log_{10}(P\text{-value}) = 4.90$], C8:0 [$-\log_{10}(P\text{-value}) = 6.28$], C10:0 [$-\log_{10}(P\text{-value}) = 4.03$], and C12:0 [$-\log_{10}(P\text{-value}) = 1.33$]. QTagSNP2 was BovineHD1700009150 (rs135934524) located at 32.90 Mbp, with MAF of 0.44. QTagSNP2 was associated with C6:0 [$-\log_{10}(P\text{-value}) = 2.76$], C8:0 [$-\log_{10}(P\text{-value}) = 3.27$], and C10:0 [$-\log_{10}(P\text{-value}) = 2.24$]. QTagSNP1 and QTagSNP2 showed the strongest associations with C8:0, and LD between QTagSNP1 and QTagSNP2 was $r^2 = 0.04$.

For summer milk samples, QTagSNP1 was BovineHD1700008490 (rs109290136) located at 30.08 Mbp (Figure 1B), with MAF of 0.44. QTagSNP1 was associated with C6:0 [$-\log_{10}(P\text{-value}) = 6.82$], C8:0 [$-\log_{10}(P\text{-value}) = 7.93$], C10:0 [$-\log_{10}(P\text{-value}) = 6.13$], and C12:0 [$-\log_{10}(P\text{-value}) = 3.35$]. QTagSNP2 was BovineHD1700008967 (rs135465158) located at 32.17 Mbp (Figure 1C), with MAF of 0.14. QTagSNP2 was associated with C6:0 [$-\log_{10}(P\text{-value}) = 2.82$], C8:0 [$-\log_{10}(P\text{-value}) = 3.19$], and C10:0 [$-\log_{10}(P\text{-value}) = 1.84$]. QTagSNP1 and QTagSNP2 showed the strongest associations with C8:0. The LD between QTagSNP1 and QTagSNP2 was $r^2 = 0.07$. The LD between QTagSNP1 and all other markers in the fine-mapped region as well as significance of association with C8:0 is represented in Supplemental Figure S2A (<http://dx.doi.org/10.3168/jds.2014-8178>), and LD between QTagSNP2 and all other markers in the fine-mapped region as well as significance of association with C8:0 is represented in Supplemental Figure S2B (<http://dx.doi.org/10.3168/jds.2014-8178>).

Linkage disequilibrium between QTagSNP1 for winter milk samples and QTagSNP1 for summer milk samples was $r^2 = 0.56$; LD among other combinations of QTagSNP based on winter or summer milk samples was low ($r^2 < 0.10$).

For both winter and summer milk samples, 2 QTagSNP were identified. These 2 QTagSNP were used for

haplotype construction, resulting in 4 haplotypes. As QTagSNP were not the same in winter and summer milk samples, different haplotypes were constructed for each season.

Predicted Effects of Haplotypes

Predicted effects of haplotypes are shown in Table 2. For winter samples, frequencies of haplotypes were 0.33 for A-A, 0.21 for A-G, 0.12 for C-A, and 0.35 for C-G. Whereas A-A haplotypes were associated with higher contents of C6:0, C8:0, C10:0, C12:0, C14:0, and the index C6:0–C14:0, C-G haplotypes were associated with lower contents of these FA and index. The absolute difference between one copy of the most contrasting haplotypes (A-A and C-G) was 0.040 for C6:0, 0.039 for C8:0, 0.090 for C10:0, 0.054 for C12:0, 0.065 for C14:0, and 0.239 for the index C6:0–C14:0. The fraction of genetic variance explained by haplotypes was 2.7% for C6:0, 2.8% for C8:0, 1.4% for C10:0, 0.5% for C12:0, 0.3% for C14:0, and 0.7% for the index C6:0–C14:0.

In winter samples, predicted effects of the A-A haplotype differed significantly ($P \leq 0.05$; Table 2) from effects of A-G, C-G, and C-A haplotypes for C6:0, C8:0, and C10:0. Effects of the C-G haplotype differed significantly ($P \leq 0.05$) from effects of the A-G haplotype for C6:0, C8:0, and C10:0. Effects of the C-A haplotype did not differ from effects of the A-G haplotype for C6:0,

Table 2. Predicted effects of haplotypes (frequency given in parentheses after each haplotype) for de novo synthesized milk FA based on 1,640 winter milk samples and 1,581 summer milk samples

Trait	Winter milk samples				$\sigma_{haplo}^2 / \sigma_a^2$ (%) ¹
	A-A (0.33)	A-G (0.21)	C-A (0.12)	C-G (0.35)	
C4:0	0.000 ± 0.000 ^a	0.000 ± 0.000 ^a	0.000 ± 0.000 ^a	0.000 ± 0.000 ^a	0.0
C6:0	0.021 ± 0.010 ^a	0.002 ± 0.010 ^b	−0.004 ± 0.011 ^{bc}	−0.019 ± 0.010 ^c	2.7
C8:0	0.020 ± 0.009 ^a	0.002 ± 0.009 ^b	−0.002 ± 0.010 ^b	−0.019 ± 0.009 ^c	2.8
C10:0	0.045 ± 0.023 ^a	0.005 ± 0.023 ^b	−0.005 ± 0.025 ^{bc}	−0.045 ± 0.023 ^c	1.4
C12:0	0.026 ± 0.020 ^a	0.004 ± 0.020 ^{ab}	0.001 ± 0.022 ^{ab}	−0.028 ± 0.020 ^b	0.5
C14:0	0.037 ± 0.028 ^a	0.003 ± 0.028 ^{ab}	−0.012 ± 0.031 ^{ab}	−0.028 ± 0.028 ^b	0.3
C6:0–C14:0	0.106 ± 0.084 ^a	0.027 ± 0.085 ^{ab}	0.000 ± 0.091 ^{ab}	−0.133 ± 0.084 ^b	0.7
Trait	Summer milk samples				
	A-G (0.44)	A-A (0.12)	C-A (0.01)	C-G (0.42)	
C4:0	−0.009 ± 0.009 ^a	0.002 ± 0.010 ^a	0.006 ± 0.011 ^a	0.000 ± 0.009 ^a	0.3
C6:0	−0.043 ± 0.021 ^a	−0.009 ± 0.022 ^b	0.046 ± 0.027 ^c	0.005 ± 0.021 ^{bc}	9.7
C8:0	−0.035 ± 0.016 ^a	−0.003 ± 0.017 ^b	0.030 ± 0.021 ^b	0.008 ± 0.016 ^b	9.0
C10:0	−0.068 ± 0.031 ^a	0.003 ± 0.033 ^b	0.030 ± 0.043 ^b	0.034 ± 0.032 ^b	3.5
C12:0	−0.049 ± 0.032 ^a	0.003 ± 0.035 ^{ab}	−0.006 ± 0.045 ^{ab}	0.052 ± 0.033 ^b	1.8
C14:0	−0.055 ± 0.039 ^a	0.004 ± 0.044 ^{ab}	0.000 ± 0.054 ^{ab}	0.051 ± 0.041 ^b	0.9
C6:0–C14:0	−0.329 ± 0.168 ^a	−0.050 ± 0.180 ^b	0.213 ± 0.237 ^b	0.166 ± 0.172 ^b	5.0

^{a–c}For each trait (i.e., within a row), different letters indicate a significant difference between haplotypes at $P \leq 0.05$, using Student's *t*-test.

¹ $\sigma_a^2 = \sigma_{a*}^2 + \sigma_{haplo}^2$, where σ_a^2 is the total additive genetic variance, σ_{a*}^2 is the additive genetic variance that remains after accounting for haplotype effects, and σ_{haplo}^2 is the haplotype variance.

C8:0, and C10:0, but did differ significantly ($P \leq 0.05$) from effects of the C-G haplotype for C8:0. These results suggest that 2 groups of haplotypes with distinct effects (A-A and A-G/C-A/C-G) exist for C6:0 and C10:0, and that 3 groups of haplotypes with distinct effects (A-A, C-G, and A-G/C-A) exist for C8:0.

For summer samples, frequencies of haplotypes were 0.44 for A-G, 0.12 for A-A, 0.01 for C-A, and 0.42 for C-G. Whereas C-G haplotypes were associated with higher contents of C6:0, C8:0, C10:0, C12:0, C14:0, and the index C6:0–C14:0, A-G haplotypes were associated with lower contents of these FA and index. The absolute difference between one copy of the most contrasting haplotypes (C-G and A-G) was 0.048 for C6:0, 0.043 for C8:0, 0.102 for C10:0, 0.101 for C12:0, 0.106 for C14:0, and 0.495 for the index C6:0–C14:0. The fraction of genetic variance explained by haplotypes was 0.3% for C4:0, 9.7% for C6:0, 9% for C8:0, 3.5% for C10:0, 1.8% for C12:0, 0.9% for C14:0, and 5.0% for the index C6:0–C14:0.

In summer samples, predicted effects of the A-G haplotype differed significantly ($P \leq 0.05$; Table 2) from effects of A-A, C-G, and C-A haplotypes for C6:0, C8:0, C10:0, and the index C6:0–C14:0. Additionally, effects of the A-G haplotype differed significantly ($P \leq 0.05$) from effects of the C-G haplotype for C12:0 and C14:0. Effects of the C-G haplotype did not differ from the effects of C-A and A-A haplotypes for any of the traits. These results suggest that 2 groups of haplotypes have distinct effects (A-G and A-A/C-A/C-G) for C6:0, C8:0, C10:0, C12:0, C14:0, and the index C6:0–C14:0.

DISCUSSION

In the present study, we refined the location of a QTL first described by Bouwman et al. (2012). This QTL seems to influence multiple de novo synthesized FA. We fine-mapped BTA17 by using imputed 777K SNP genotypes and by using winter and summer milk FA composition. To further characterize the effects associated with this genomic region, we constructed haplotypes for each season.

Fine-Mapping of BTA17

The fine-mapping of BTA17 combined high-density SNP genotyping with imputation. Imputation was based on a large reference population genotyped with 777K SNP. Additionally, the 55 sires belonging to our experimental population were genotyped with both 50K and 777K SNP arrays. Our experimental population, which was composed of the daughters of the 55 sires, was imputed from 50K to 777K SNP genotypes using Beagle software (Browning and Browning, 2009). The

estimated error of this imputation was <1%. Pausch et al. (2013) showed that imputation to high-density genotypes largely depends on the size of the reference population. An imputation accuracy of about 99% can be obtained when a reference population of more than 400 animals is used (Pausch et al., 2013). This is in line with the imputation accuracy obtained in the current study. When imputation accuracy is high, GWAS based on imputed genotypes can assist in fine-mapping because imputation provides a high-resolution view of an associated region and increases the chance that a causal SNP can be directly identified (Marchini and Howie, 2010). In the present study, the number of SNP increased by at least 10 times with the imputation of BTA17 from 50K to 777K SNP genotypes.

The GWAS by Bouwman et al. (2012) with 50K SNP genotypes identified a QTL associated with milk FA composition on BTA17. By fine-mapping BTA17 with the imputed 777K SNP genotypes, additional SNP were found to be significantly associated with milk FA, and these were more significant than the SNP found by Bouwman et al. (2012). In addition, multiple FA showed associations with the same genomic region on BTA17, in both winter and summer milk samples (Supplemental Figure S1; <http://dx.doi.org/10.3168/jds.2014-8178>). We focused on the strongest and most consistent associations found in winter and summer milk samples. These associations were identified in a region located between 29 and 34 Mbp. Additional analyses in which we extended the region (26–34 Mbp) showed results comparable to those presented herein.

Within this genomic region, summer milk showed more pronounced associations than winter milk samples. Duchemin et al. (2013) reported strong genetic correlations between winter and summer milk fat composition of de novo synthesized FA (e.g., 0.95 for C6:0, 0.93 for C8:0, and 0.95 for C10:0). These strong genetic correlations suggest that de novo FA in winter and in summer milks are genetically the same trait. In addition, GWAS by Bouwman et al. (2012) showed that many genomic regions associated with milk FA in winter milk could be confirmed in summer milk samples (e.g., BTA17). Therefore, it is likely that milk FA composition is influenced by similar groups of genes. When studying the effects of *DGAT1* polymorphism on milk fat composition in winter and summer milk samples, Duchemin et al. (2013) concluded that the genotypic effects were in the same direction, but that some of the genotypic effects were larger in summer compared with winter. Duchemin et al. (2013) concluded that differences between winter and summer milk fat composition were likely due to differences in the diets of the cows and that the effects of *DGAT1* were scaled. This scaling resulted in a significant *DGAT1* by season interaction,

especially for short-chain FA (C4:0 to C14:0). In the present study, similar scaling effects might explain the more pronounced associations found in summer compared with winter milk samples.

Construction of Haplotypes

Haplotypes were constructed by first retrieving the most significant SNP within the fine-mapped region. This SNP, QTagSNP1, was associated with C8:0. Most of the variation in the region was explained by QTagSNP1, but not all. The remaining variation was accounted for by QTagSNP2 (results shown for summer samples, Figure 1B and 1C). After adjusting for both QTagSNP, no other significant SNP was found. Based on the 2 QTagSNP, 4 haplotypes were constructed. In summer milk samples, these haplotypes explained almost 10% of the genetic variance in C6:0, 9% in C8:0, 3.5% in C10:0, 1.8% in C12:0, and 0.9% in C14:0 (Table 2). When these FA were combined into an index, haplotypes explained 5% of the genetic variance in de novo synthesized milk FA (C6:0–C14:0; Table 2). After testing for differences between these haplotypes, we concluded that estimated effects in summer milk for 3 out of 4 haplotypes did not differ from each other. Therefore, our 4 haplotypes could be divided in 2 groups with distinct effects on C6:0, C8:0, C10:0, C12:0, C14:0, and the index C6:0–C14:0: A–G versus the remaining haplotypes. The existence of 2 groups of haplotypes with distinct effects could be explained by 1 causal variant; that is, 1 QTL. However, we cannot exclude the presence of multiple causal variants in strong LD.

The QTL region is associated with multiple de novo synthesized FA. De novo synthesis occurs within the mammary gland of a cow and is a process that elongates precursors by adding C2:0. These precursors originate from blood lipids and can be acetate (C2:0), propionate (C3:0), or butyrate (C4:0). Butyrate in milk may originate from de novo synthesis or directly from β -hydroxybutyrate derived from the blood (e.g., Craninx et al., 2008). Depending on the precursor, the elongation process ends at either C16:0 or C17:0. Results of the current study show that predicted effects of haplotypes increase from C6:0 to C14:0; however, the proportion of genetic variance explained by haplotypes decreases from C6:0 to C14:0. This increase of haplotype effects tends to be more pronounced in summer than in winter milk samples (Table 2). These results suggest that our candidate gene is involved in the elongation of FA or the early termination of this process (Barber et al., 1997), and it might be upregulated in summer compared with winter milk samples.

Interestingly, in other species, such as humans, macaques, and pigs, this genomic region is highly con-

served. Further, in dairy cattle breeds, 2 studies suggested that this region on BTA17 contains signatures of selection: Qanbari et al. (2011) identified signatures of selection in a region close to the *PGRMC2* gene (progesterone receptor membrane component 2) at 29.8 Mbp; and Stella et al. (2010) in a region close to the *SPRY1* gene [sprout homolog1, antagonist of FGF signaling (*Drosophila*)] at 34.7 Mbp. This genomic region might be related to a highly conserved evolutionary mechanism.

Candidate Genes

Information on candidate genes possibly associated with de novo synthesized FA was retrieved from the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>). The QTL region on BTA17 contains 29 genes, but 18 of these have not yet been characterized. Between QTagSNP1 and QTagSNP2 in summer samples are 11 genes, of which 5 have been characterized (Figure 1A).

The characterized gene that is closest to the most significant association is *PGRMC2*, which is located between 29.87 and 29.89 Mbp. This gene belongs to the superfamily cytochrome b5-like heme/steroid binding domain. This superfamily is involved in the FA metabolic process and oxido-reductase activity. In humans, this gene has been associated with breast adenocarcinoma (Causey et al., 2011), and it was identified as a regulator of cytochrome P450 enzyme activity (Wendler and Wehling, 2013). By sequencing the mRNA found in the milk fat layer, Lemay et al. (2013) showed that *PGRMC2* is expressed in humans throughout lactation, including in colostrum, transitional, and mature milk.

In cattle, *PGRMC2* has been associated with fertility. Kowalik et al. (2013) showed that expression of *PGRMC2* mRNA in the bovine endometrium was higher in the first trimester of pregnant cows compared with cyclic animals. However, the translation of *PGRMC2* mRNA into protein within the bovine endometrium was not different between cyclic and pregnant cows. In our study, cows in the winter and summer sampling periods were in different stages of lactation (average of 166 d in winter and average of 247 d in summer samples) and probably at different stages of pregnancy. This might explain the more pronounced associations found in summer milk samples. Therefore, we performed additional analyses in which we investigated interactions between stage of lactation and our QTagSNP in both seasons. None of these interactions was significant (results not shown). Bionaz et al. (2012) showed that *PGRMC2* is expressed during lactation in bovine mammary tissue, although *PGRMC2* has not been associated with milk FA composition in dairy cattle.

Of the genes located within our QTL region, Bionaz et al. (2012) showed that 4 other genes are highly expressed during lactation in bovine mammary tissue: UPF0462 protein C4orf33-like (*LOC513251*), sodium channel and clathrin linker 1 (*SCLT1*), la-related protein 1B-like (*LOC515517*), and chromosome 17 open reading frame, human C4orf29 (*C17H4orf29*). The location for these genes is between 29.10 and 29.12 Mbp for *LOC513251*, between 29.12 and 29.35 Mbp for *SCLT1*, between 30.03 and 30.07 Mbp for *LOC515517*, and between 30.10 and 30.13 Mbp for *C17H4orf29*. By sequencing the mRNA found in milk fat layer, Lemay et al. (2013) showed in humans that 3 of these 4 genes—*C17h4orf33* (validated *LOC513251* gene in humans), *LARP1B* (validated *LOC515517* gene in humans), and *C17H4orf29*—are expressed during all stages of lactation. These 4 genes have not yet been associated with milk FA composition.

In the present paper, we refined the location of a QTL associated with multiple de novo synthesized milk FA to a region between 29.0 and 34.0 Mbp on BTA17. We characterized the effects associated with this region by constructing haplotypes and identified candidate genes possibly related to this QTL.

CONCLUSIONS

The fine-mapping of BTA17 improved the location of a QTL associated with multiple de novo synthesized milk FA. In summer milk samples, this QTL region explained a large proportion of the genetic variance in these FA individually (e.g., 10% in C6:0). When all de novo synthesized milk FA were combined into an index, this QTL region explained 5% of the genetic variance. This QTL region seems to be involved in either the elongation process of the de novo FA synthesis or in the early termination of this process. In addition, the effects of this QTL region were greater in summer compared with winter milk samples. Candidate genes associated with milk FA composition could not be clearly identified for this QTL because the QTL region on BTA17 is still being characterized. A characterized gene that might be of interest within the QTL region is *PGRMC2*.

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