Joint genome-wide association study for milk fatty acid traits in Chinese and Danish Holstein populations

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ABSTRACT

The identification of causal genes or genomic regions associated with fatty acids (FA) will enhance our understanding of the pathways underlying FA synthesis and provide opportunities for changing milk fat composition through a genetic approach. The linkage disequilibrium between adjacent markers is highly consistent between the Chinese and Danish Holstein populations, such that a joint genome-wide association study (GWAS) can be performed. In this study, a joint GWAS was performed for 16 milk FA traits based on data of 784 Chinese and 371 Danish Holstein cows genotyped by a high-density bovine single nucleotide polymorphism (SNP) array. A total of 486,464 SNP markers on 29 bovine autosomes were used. Bonferroni corrections were applied to adjust the significance thresholds for multiple testing at the genome- and chromosome-wide levels. According to the analysis of either the Chinese or Danish data individually, the total numbers of overlapping SNP that were significant at the chromosome level were 94 for C14:1, 208 for the C14 index, and 1 for C18:0. Joint analysis using the combined data of the 2 populations detected greater numbers of significant SNP compared with either of the individual populations alone for 7 and 10 traits at the genome- and chromosome-wide significance levels, respectively. Greater numbers of significant SNP were detected for C18:0 and the C18 index in the Chinese population compared with the joint analysis. Sixty-five significant SNP across all traits had significantly different effects in the 2 populations. Ten FA were influenced by a quantitative trait loci (QTL) region including *DGAT1*. Both C14:1 and the C14 index were influenced by a QTL region including *SCD1* in the combined population. Other QTL regions also showed significant associations with the studied FA. A large region (14.9–24.9 Mbp) in BTA26 significantly influenced C14:1 and the C14 index in both populations,

mostly likely due to the SNP in *SCD1*. A QTL region (69.97–73.69 Mbp) on BTA9 showed a significantly different effect on C18:0 between the 2 populations. Detection of these important SNP and the corresponding QTL regions will be helpful for follow-up studies to identify causal mutations and their interaction with environments for milk FA in dairy cattle.

Key words: fatty acid, genome-wide association, Chinese and Danish Holstein, dairy cow

INTRODUCTION

Fat is an important nutrient component of dairy products, and the FA composition differs according to carbon length and degree of saturation. Short- and medium-chain saturated FA are synthesized *de novo* in the mammary gland, whereas long-chain FA are derived from circulating plasma lipids (Harfoot and Hazlewood, 1997). Short- and medium-chain FA have moderate heritability (~ 0.4), and long-chain FA have low to moderate heritability (0.1–0.3; Stoop et al., 2008; Krag et al., 2013). Identification of causal genes or genomic regions will enhance our understanding of FA synthesis pathways and increase the possibility of changing milk fat composition by a genetic approach to meet economic and human health needs.

Mutations of *DGAT1* and *SCD1* have important effects on milk fat composition (Mele et al., 2007; Schenink et al., 2007, 2008; Conte et al., 2010). However, the synthesis of milk fat is regulated by many genes, and much of the variation in milk fat composition cannot be attributed to only *DGAT1* and *SCD1* (Bionaz and Loor, 2008). With the recent developments of high-throughput SNP genotyping and genome sequencing technologies, genome-wide association studies (GWAS) have become widely accepted as a primary research approach to analyze complex traits; GWAS provide increased opportunities to investigate the genetic architecture of milk fat composition. The first GWAS of milk FA in Dutch dairy cattle (Bouwman et al., 2011) showed that from 2.2 to 61.9% and from 3.3 to 67.8% of genetic variations in some milk FA could

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be explained by *DGAT1* and *SCD1*, respectively. Other regions also contribute to the genetic variation in milk composition.

Genome-wide association studies for FA in different populations can detect common and population-specific QTL and increase the understanding of causal genes that influence FA synthesis. Combining data from different populations to enhance the power of identifying significant SNP is especially important for traits that are expensive to measure, such as FA. Zhou et al. (2013) reported high consistency of the linkage disequilibrium (**LD**) between adjacent markers between the Chinese and Danish Holstein populations. Therefore, it is reasonable to assume that both populations segregate for the same causative variants, which offers a good opportunity to perform a joint GWAS using both populations.

In a joint analysis of populations, some important SNP may be undetected due to different or even opposite effects within the populations. Previous studies observed a “SNP genotype $\times$ environment interaction,” in which SNP showed different effects at different environmental levels (Lillehammer et al., 2008, 2009; Hayes et al., 2009). As the production environments differ between the Chinese and Danish Holstein populations, it would be interesting to investigate whether significant differences in SNP effects occur between the production systems.

The present study had 2 main objectives. The first was to perform a joint GWAS of the Chinese and Danish Holstein populations by using a high-density (**HD**) chip to detect QTL affecting 16 milk FA traits with high power. The second objective was to investigate SNP with similar and different effects between the populations.

MATERIALS AND METHODS

Phenotypes

The data set consisted of 784 Chinese and 371 Danish Holstein cows. All Chinese cows were housed in 18 herds and were within parity 1 to 6. Most of these cows were between 60 and 600 d in milk. Danish cows were distributed in 20 herds, in mid lactation (d 129 to 229), and within parity 1 to 3. Fat compositions of collected milk samples were measured independently in each country. Milk FA were measured once per cow by GC and expressed in terms of the weight proportion of total fat weight. Details about the measurement of FA can be found in Li et al. (2014) for Chinese cows and Poulsen et al. (2012) for Danish cows.

In total, 13 milk FA were measured in both populations: even-chain saturated FA (C8:0–C18:0), odd-chain

saturated FA (C15:0), and unsaturated FA [C14:1, C16:1, C18:1n-9, C18:2n-6, C18:3n-3, and C18:2 *cis*-9,*trans*-11 (**CLA911**)]. Three FA indices were also analyzed: the unsaturation indices for C14 (C14 index), C16 (C16 index), and C18 (C18 index). These indices were not directly measured but were calculated using the following formulas (Kelsey et al., 2003):

$$\text{C14 index} = \text{C14:1}/(\text{C14:1} + \text{C14:0}) \times 100,$$

$$\text{C16 index} = \text{C16:1}/(\text{C16:1} + \text{C16:0}) \times 100, \text{ and}$$

$$\text{C18 index} = \text{C18:1n-9}/(\text{C18:1n-9} + \text{C18:0}) \times 100.$$

For the 13 directly measured FA traits, measurements outside the range of $\mu \pm 3.5\sigma$ (mean $\pm$ 3.5 SD) for each trait in each country were removed. Table 1 presents the descriptive statistics of FA.

Genotypes

The 371 Danish cows were genotyped by using the BovineHD BeadChip (Illumina Inc., San Diego, CA) including 777,962 markers. All animals met the criteria of a minimum call rate of 80% and average GenCall scores above 0.65. The 784 Chinese cows were genotyped by using the BovineSNP50 BeadChip (Illumina Inc.) containing 54,001 SNP. The Beagle software package (Browning and Browning, 2009) was used to impute the Chinese cows to HD marker data by using 96 HD-genotyped Chinese bulls as the reference population. Single nucleotide polymorphisms with unknown positions or on the X chromosome were excluded from the study. Marker loci with minor allele frequencies $<1\%$ within each population were excluded. The final marker set included 486,464 SNP on BTA in both populations. The SNP positions were based on the UMD3.1 assembly (ftp://ftp.cbcb.umd.edu/pub/data/assembly/Bos_taurus/Bos_taurus_UMD_3.1/).

Statistical Analysis

Three single SNP linear regression models were used to perform GWAS for detecting QTL associated with FA. The first model was used to analyze data of the Chinese Holstein and the Danish Holstein separately:

$$y_{ijk} = \text{herd}_i + \text{parity}_j + b_1 \times \text{dim} + b_2 \times e^{-0.05 \times \text{dim}} + qX_k + \alpha_k + e_{ijk}, \quad [1]$$

where y is the phenotype; herd is the fixed effect of herd; parity is the fixed effect of parity; dim is the number of days in milk; b_1 and b_2 are the regression

coefficients of y on dim and its function $e^{-0.05 \times \text{dim}}$, respectively; x is the genotype of a SNP in the individual, coded as 0, 1, or 2 for genotype A_1A_1 , A_1A_2 , or A_2A_2 respectively; q is the regression coefficient of y on x (i.e., SNP effect); α is the random additive genetic effect with $\alpha \sim N(0, \mathbf{A}\sigma_a^2)$, where $\mathbf{A}$ is the pedigree-based additive genetic relationship matrix and σ_a^2 is the additive genetic variance; and e is the random residual with $e \sim N(0, \mathbf{I}\sigma_e^2)$, where $\mathbf{I}$ is the identity matrix and σ_e^2 is the residual variance.

The second model was used for the joint analysis of both populations, assuming that the SNP effect was the same in both populations:

$$y_{\text{hijk}} = \text{country}_h + \text{herd}_i + \text{parity}_j + b_1 \times \text{dim}(\text{country}_h) + b_2 \times e^{-0.05 \times \text{dim}}(\text{country}_h) + qx_k + \alpha_k + e_{\text{hijk}}, \tag{2}$$

where y , herd , parity , dim , q , x , and α are the same as in model [1], but country is a fixed effect in model [2]. We assumed that the 2 populations had different regressions on dim and the function of dim , as well as different residual variances.

Accordingly, $e \sim N(0, \mathbf{D}\sigma_e^2)$, where $\mathbf{D}$ is a diagonal matrix with diagonal element $d_{ii} = 1/w_i$ and σ_e^2 is the residual variance. The weight w_i was used to account for the different residual variances in the populations; thus, w_i was the same for all records within the same population but differed between populations. Weights w_{DK} for the Danish population and w_{CN} for the Chinese

population were calculated as follows: $w_{CN} = \sigma_{e_{DK}}^2 / \sigma_{e_{CN}}^2$ and $w_{DK} = 1$; therefore, w_{CN} was calculated by $w_{CN} / \bar{w}$, and w_{DK} was calculated by $1 / \bar{w}$, where $\bar{w} = (n1 \times w_{CN} + n2) / (n1 + n2)$; and $n1$ and $n2$ are the number of records for the Chinese and the Danish population, respectively.

The third model allowed us to account for different effects of the SNP in the populations:

$$y_{\text{hijk}} = \text{country}_h + \text{herd}_i + \text{parity}_j + b_1 \times \text{dim}(\text{country}_h) + b_2 \times e^{-0.05 \times \text{dim}}(\text{country}_h) + (q + \Delta q \times E)x_k + \alpha_k + e_{\text{hijk}}, \tag{3}$$

where the variables were defined as in model 2, except for the term $(q + \Delta q \times E)x_k$. In this term, q is the SNP effect in the Danish Holstein population; Δq is the difference in the SNP effect between the populations; and E is the country indicator ($= 0$ for Danish and $= 1$ for Chinese Holstein populations). Thus, the SNP effect in the Chinese Holstein population was $q + \Delta q$.

Significance Test

Testing was done by a Wald test with a null hypothesis (H_0) as follows: $q = 0$ for model [1] and model [2]; and $q = 0$ or $\Delta q = 0$ for model [3]. All analyses were performed in the DMU package (Madsen et al., 2010).

Stringent significance thresholds were determined by using Bonferroni multiple testing corrections. The genome-wide significance threshold was obtained by dividing the nominal significance threshold (0.05) by the

Table 1. Descriptive statistics of milk FA traits in Chinese and Danish Holstein populations

Trait	Chinese Holstein					Danish Holstein				
	N	Mean	SD	Maximum	Minimum	N	Mean	SD	Maximum	Minimum
C8:0	784	0.58	0.22	1.20	0.13	371	1.46	0.23	2.04	0.75
C10:0	784	2.19	0.43	3.29	0.97	371	3.15	0.57	4.60	1.58
C12:0	784	2.87	0.54	4.51	1.19	371	3.56	0.66	5.32	2.00
C14:0	784	9.89	1.28	13.55	5.81	371	11.25	1.29	14.48	6.89
C14:1	784	0.83	0.22	1.60	0.34	370	0.97	0.27	1.81	0.28
C15:0	780	0.97	0.15	1.49	0.46	369	1.10	0.19	1.72	0.67
C16:0	781	32.67	1.95	38.66	26.79	371	28.86	3.24	40.08	21.34
C16:1	779	1.65	0.35	2.85	0.90	370	1.49	0.36	2.74	0.39
C18:0	784	12.17	1.76	17.37	7.40	370	10.45	2.00	17.48	5.87
C18:1n-9	784	28.57	2.81	38.29	18.87	371	20.07	2.97	29.21	12.36
C18:2n-6	782	4.00	0.45	5.53	2.45	370	1.69	0.28	2.66	1.01
C18:3n-3	781	0.42	0.06	0.63	0.24	371	0.49	0.10	0.76	0.26
CLA911 ¹	782	0.40	0.09	0.77	0.20	370	0.62	0.16	1.09	0.31
C14 index ²	784	7.76	1.68	14.62	3.46	370	7.88	1.92	14.09	2.88
C16 index ³	777	4.83	1.02	9.12	0.79	370	4.90	1.03	8.67	1.34
C18 index ⁴	784	70.14	3.33	80.30	55.89	370	65.81	4.18	77.11	50.68

¹CLA911 = C18:2 *cis*-9,*trans*-11.
²C14 index = C14:1/(C14:1 + C14:0) × 100.
³C16 index = C16:1/(C16:1 + C16:0) × 100.
⁴C18 index = C18:1n-9/(C18:1n-9 + C18:0) × 100.

total number of SNP (486,464) included in the analysis. The P -value after Bonferroni multiple testing corrections was about $1.03e^{-7}$ (i.e., $-\log_{10}(P\text{-value}) = 7.00$). However, the Bonferroni correction ignores the fact that tests are correlated due to LD between markers and, therefore, leads to overcorrection. This problem will be exacerbated as the marker density increases (Han et al., 2009).

Therefore, a less-stringent chromosome-wide significance threshold was considered; namely, a threshold equal to 0.05 divided by the number of SNP on a chromosome. The number of SNP varied from 9,233 on BTA25 to 29,789 on BTA1. Thus, the chromosome-wide significance threshold values ranged from $5.42e^{-6}$ [$-\log_{10}(P\text{-value}) = 5.27$] to $1.68e^{-6}$ [$-\log_{10}(P\text{-value}) = 5.77$].

Defining QTL Regions

Normally, QTL regions are defined subjectively. Multiple SNP within or near a QTL region are expected to yield significant results in a single SNP analysis, because many SNP that are physically located near the causal variant will tend to be in LD. In this study, from one chromosome-wide significant SNP, the region was extended as long as another significant SNP occurred within 1.5 Mbp of the previous one. The region ended at the last significant SNP that was not followed by another significant SNP within the next 1.5 Mbp. A QTL region that contained at least 2 significant SNP was called a “real QTL region” (Bouwman et al., 2011).

Consistencies of Allele Frequency and LD Between Populations

The software Beagle (Browning and Browning, 2009) was used to phase each chromosome and impute all missing genotypes in each population separately. Consistencies of the allele frequency and LD between the Holstein populations were assessed for each small region. Three sizes of regions (50, 100, and 200 markers) were considered. If the last region of a chromosome included less than the required number of markers, then this region was merged with the previous region. For each region, the consistency of allele frequency was measured by correlating the allele frequencies between the 2 populations. The consistency of LD phase was measured by correlating the r_{LD} (a measure of LD) of all marker pairs within the region between the 2 populations (de Roos et al., 2008; Zhou et al., 2013). The r_{LD} was calculated as follows:

$$r_{LD} = \frac{p(A_1B_1) - p(A_1)p(B_1)}{\sqrt{p(A_1)p(A_2)p(B_1)p(B_2)}},$$

where $p(A_1B_1)$ is the observed frequency of haplotypes with allele 1 at marker locus A and allele 1 at marker locus B, $p(A_{1(2)})$ is the frequency of allele 1 or 2 at marker locus A, and $p(B_{1(2)})$ is the frequency of allele 1 or 2 at marker locus B (Hill and Robertson, 1968). The square of r_{LD} is a popular measure of LD within a population. Consistency of LD between populations is often measured as correlation of r_{LD} instead of correlation of r_{LD}^2 , because different signs (+ or -) of r_{LD} indicate different LD phases.

RESULTS

Descriptive Statistics

Table 1 presents the descriptive statistics of the 16 milk FA traits. Number of records differed among the traits due to the removal of different numbers of outliers (outside the range of $\mu \pm 3.5\sigma$). Numbers of outliers for the 16 traits ranged from 0 to 5 in the Chinese population and from 0 to 2 in the Danish population. For C16:0, C16:1, C18:0, C18:1n-9, C18:2n-6, and the C18 index, the average values of phenotypes of the Chinese population were higher than those of the Danish population. For the remaining 10 FA traits, the average values of the Chinese population were lower. In addition, the Danish population had larger variations for all traits except C18:2n-6.

GWAS Using Data of Each Population Separately

We performed GWAS for the 16 FA traits in the Chinese and Danish Holstein populations separately. Table 2 presents the numbers of significant SNP detected for all analyzed FA traits at the genome- and chromosome-wide significance levels. Detailed information about these SNP can be found in Supplementary Tables S1 and S2 (<http://dx.doi.org/10.3168/jds.2015-9383>).

For the Chinese population, the numbers of SNP detected at the genome-wide level were 167 for C14:1, 235 for the C14 index, 8 for C18:0, and 17 for the C18 index. At the chromosome-wide level, there were greater numbers of significant SNP for these 4 traits, as well as 8 significant SNP for C10:0, 8 for C16:0, 10 for C16:1, 10 for C18:1n-9, 18 for C18:2n-6, and 6 for the C16 index. Most of the significant SNP, mainly located on BTA11, BTA14, BTA20, BTA24, and BTA26, were associated with C14:1, C18:0, and the C14 and C18 indices.

For the Danish population, numerous genome-wide significant SNP were detected for C14:1 (59 SNP) and the C14 index (175 SNP). In contrast to the Chinese Holstein population, no significant effects were found

Table 2. Numbers of significant SNP associated with 16 milk FA traits in the Chinese (CN), Danish (DK), and combined (CN+DK) populations

Trait	Genome-wide level			Chromosome-wide level		
	CN	DK	CN+DK	CN	DK	CN+DK
C8:0	0	0	0	0	3	1
C10:0	1	0	0	8	0	6
C12:0	0	0	0	2	0	0
C14:0	0	0	0	1	0	2
C14:1	167	59	327	312	122	521
C15:0	0	0	0	0	0	6
C16:0	0	0	0	8	2	15
C16:1	0	12	19	10	30	51
C18:0	8	0	1	146	1	60
C18:1n-9	0	0	4	10	0	40
C18:2n-6	1	0	2	18	6	13
C18:3n-3	0	0	0	0	2	4
CLA911 ¹	0	0	0	0	5	10
C14 index ²	253	175	461	406	287	709
C16 index ³	0	0	2	6	12	34
C18 index ⁴	17	0	14	173	5	121

¹CLA911 = C18:2 *cis*-9,*trans*-11.

²C14 index = $C14:1 / (C14:1 + C14:0) \times 100$.

³C16 index = $C16:1 / (C16:1 + C16:0) \times 100$.

⁴C18 index = $C18:1n-9 / (C18:1n-9 + C18:0) \times 100$.

for C18:0 or the C18 index in the Danish Holstein population, whereas 12 genome-wide significant SNP were found for C16:1 in the Danish population. At the chromosome-wide level, there were significant SNP for C8:0 (3 SNP), C16:0 (2 SNP), C18:2n-6 (6 SNP), C18:3n-3 (2 SNP), CLA911 (5 SNP), the C16 index (12 SNP), and the C18 index (5 SNP). Significant SNP detected in Danish cows were mainly located on BTA14, BTA17, BTA18, and BTA26, and most of them were associated with C14:1, C16:1, and the C14 index. As expected, fewer significant SNP were detected in the Danish population compared with the Chinese population.

GWAS Using Combined Data of Both Populations

Joint GWAS analysis assuming the same SNP effect in both populations (model [2]) generally presented greater numbers of significant SNP compared with either population alone (Supplementary Table S3; <http://dx.doi.org/10.3168/jds.2015-9383>). The use of the joint data at the genome-wide level detected more significant SNP for 6 traits, fewer significant SNP for 3 traits, and the same number of significant SNP for 7 traits, compared with use of the Chinese population alone. At the chromosome-wide level, more significant SNP were detected for 11 traits and fewer significant SNP for 5 traits.

Table 3 presents the distribution of overlapping significant SNP between the analyses based on each separate data set and the combined data set. Overlap-

ping SNP among the 3 data sets (Chinese data, Danish data, and joint data) mainly focused on 2 traits, C14:1 and the C14 index. We identified 94, 256, and 115 chromosome-wide significant SNP for C14:1, and 208, 394, and 267 chromosome-wide significant SNP for the C14 index in the Chinese, Danish, and joint data sets, respectively. We observed high overlap between the Chinese and joint data sets for C18:0 (35 SNP) and the C18 index (60 SNP) at the chromosome-wide significance level.

Table 4 presents the QTL regions detected for 16 milk FA traits based on the joint data set. We found 9 QTL regions influencing the C18 index, 4 regions influencing C18:0, 3 regions influencing C14:1, and 3 regions influencing the C14 index across different chromosomes. Three QTL regions were associated with 2 or more milk FA traits. Among them, an important QTL region (1.46–3.37 Mbp) on BTA14, which included *DGAT1*, was associated with 10 FA traits; a QTL region (29.63–33.04 Mbp) on BTA20, which included *GHR*, was associated with C14:1 and C18:0; and a QTL region (10.00–27.94 Mbp) on BTA26, which included *SCD1*, influenced C14:1 and the C14 index.

Comparison of the Chinese and Danish Holstein Data Sets

According to the analyses based on Chinese data and Danish data separately, overlapped significant SNP were mainly found for C14:1 and the C14 index (Table

3). A large number of significant SNP was also found for these 2 traits when using the combined data (Table 2). Details regarding these overlapping chromosome-wide significant SNP and involved genes are shown in Supplementary Table S4 (<http://dx.doi.org/10.3168/jds.2015-9383>). Manhattan plots revealed a very high peak on BTA26 in the Chinese, Danish, and combined populations (Supplementary Figures S1, S2, S3, and S4; <http://dx.doi.org/10.3168/jds.2015-9383>). The top SNP in this peak with the highest $-\log_{10}$ (P -value) was within *SCD1*. This SNP explained 8.8 and 13.0% of the total variance for C14:1, as well as 12.0 and 19.0% of the total variance for the C14 index in the Chinese and the Danish populations, respectively.

To report regions that showed agreement between the 2 populations for >1 significant SNP, we used the regions defined by significant SNP with a $-\log_{10}$ (P -value) of at least 3.0, rather than the chromosome-wide significant SNP. We detected 1 region (87.54–92.46 Mbp) on BTA3 for C12:0, 1 region (70.34–73.69 Mbp) on BTA9 for C18:0, 2 regions (39.33–43.48 Mbp and 48.15–52.61 Mbp) on BTA11 for the C18 index, 1 region (1.59–3.37 Mbp) on BTA14 for C16:0, C16:1, the C16 index, and C18:1n-9, 1 region (39.10–49.40 Mbp) on BTA14 for the C16 index, and 1 region (10.07–32.37 Mbp) on BTA26 for C14:1 and the C14 index in both populations. Similarities in detected regions were not found for C8:0, C10:0, C15:0, C18:2n-6 or C18:3n-3 (data not shown).

Table 5 presents the numbers of SNP in the Chinese and Danish populations and the numbers of SNP with

significantly different effects between the 2 populations at the chromosome-wide significance level. In the joint GWAS analysis with different SNP effects in the 2 populations (model [3]), the numbers of significant SNP were very similar to those found in analysis based on the Chinese or Danish population alone (model [1]). Across all traits, 65 SNP across all traits had significantly different effects between the 2 populations. Among these SNP, 2 SNP for C18:3n-3 and 2 SNP for CLA911 were significantly associated with C18:3n-3 and CLA911 in the Danish population. One SNP for C18:0 was significantly associated with C18:0 in both populations (Figure 1; Supplementary Table S5; <http://dx.doi.org/10.3168/jds.2015-9383>).

Within trait, 33 SNP showed significant differences for C18:0, of which 31 were located on BTA9 (69.97–73.69 Mbp). Among these 31 SNP, 21 SNP in the region from 71.76 to 71.81 Mbp were in complete LD ($r^2 = 1$, Figure 1). As shown in Figure 1, those SNP with significant differences between the 2 populations were mainly located at about 71.80 Mbp in the region 69.97–73.69 Mbp on BTA 9.

Comparing differences in regions, we detected 1 region (76.78–81.98 Mbp) on BTA8, 1 region (56.34–60.87 Mbp) on BTA11, 1 region (29.63–33.04 Mbp) on BTA20, and 1 region (11.61–14.98 Mbp) on BTA24 for C18:0 in the Chinese but not the Danish population. We also detected a large region (56.34–60.87 Mbp) on BTA11 and 8 small regions on BTA1, BTA2, BTA5, BTA12, and BTA20 for the C18 index in the Chinese but not the Danish population (data not shown).

Table 3. Overlaps of significant SNP in the Chinese (CN), Danish (DK), and combined (CN+DK) populations for 16 milk FA traits

Trait	Genome-wide level			Chromosome-wide level		
	CN vs. DK	CN vs. CN+DK	DK vs. CN+DK	CN vs. DK	CN vs. CN+DK	DK vs. CN+DK
C8:0	0	0	0	0	0	0
C10:0	0	0	0	0	2	0
C12:0	0	0	0	0	0	0
C14:0	0	0	0	0	1	0
C14:1	40	165	54	94	296	115
C15:0	0	0	0	0	0	0
C16:0	0	0	0	0	3	0
C16:1	0	0	12	0	2	19
C18:0	0	0	0	1	35	0
C18:1n-9	0	0	0	0	7	0
C18:2n-6	0	0	0	0	8	0
C18:3n-3	0	0	0	0	0	0
CLA911 ¹	0	0	0	0	0	0
C14 index ²	132	252	163	208	394	267
C16 index ³	0	0	0	0	0	4
C18 index ⁴	0	3	0	0	60	0

¹CLA911 = C18:2 *cis*-9,*trans*-11.

²C14 index = $C14:1 / (C14:1 + C14:0) \times 100$.

³C16 index = $C16:1 / (C16:1 + C16:0) \times 100$.

⁴C18 index = $C18:1n-9 / (C18:1n-9 + C18:0) \times 100$.

Table 4. Quantitative trait loci regions detected for 16 milk FA traits

Chromosome	QTL region	Start (Mbp)	End (Mbp)	Associated traits	Nearest gene	Candidate gene	Distance ¹
1	1	67.76	68.01	C18 index	<i>SEMA5B</i>	<i>SEMA5B</i>	Overlap
1	2	146.29	146.31	C18:0	LOC100337271	<i>AGPAT3</i>	400 Kb
5	1	4.27	4.31	C18 index	Unknown	Unknown	unknown
5	2	28.79	29.11	C18 index	<i>TFCP2</i>	Unknown	unknown
11	1	32.70	32.90	C18 index	<i>NRXN1</i>	Unknown	unknown
11	2	51.89	61.56	C18 index	<i>CTNNA2</i>	Unknown	unknown
12	1	69.43	70.26	C18:0	<i>DCT</i>	Unknown	unknown
14	1	1.46	3.37	C14:1, C16:0, C16:1, C18:1n-9, C18:2n-6, C18:3n-3, CLA911, C14 index, C16 index, C18 index	<i>DGAT1</i>	<i>DGAT1</i>	Overlap
14	2	65.11	65.45	C18 index	NA	Unknown	unknown
15	1	14.47	14.62	C18 index	<i>MAML2</i>	Unknown	unknown
15	2	31.02	32.77	C18 index	<i>SORL1</i>	Unknown	unknown
19	1	26.88	27.25	C15:0	<i>CXCL16</i>	<i>SREBF1</i>	8 Mbp
19	2	58.42	59.01	C16:0	<i>SLC39A11</i>	<i>FASN</i>	8 Mbp
20	1	29.63	33.04	C14:1, C18:0	<i>GHR</i>	<i>GHR</i>	Overlap
20	2	36.44	36.76	C18:1n-9	<i>WDR70</i>	<i>PRLR</i>	2 Mbp
23	1	34.82	35.91	C18:0	LOC100847951	<i>BTN1A1</i>	3.5 Mbp
26	1	10.00	27.94	C14:1, C14 index	<i>LIPJ</i> , <i>LIPK</i> , <i>ECHS1</i> , <i>SORBS1</i> , <i>NFKB2</i> , <i>CHUK</i> , <i>SCD1</i>	<i>LIPJ</i> , <i>LIPK</i> , <i>ECHS1</i> , <i>SORBS1</i> , <i>NFKB2</i> , <i>CHUK</i> , <i>SCD1</i>	Overlap
27	1	37.35	37.37	C14 index	<i>AGPAT6</i>	<i>AGPAT6</i>	1.2 Mbp

¹Distance = the physical distance between the QTL region and the candidate gene.

Figure 1. Plot of SNP effects for Chinese and Danish Holstein populations and the difference (Diff) in the region (71–72 Mbp) on BTA9 for C18:0. Color version available online.

We further investigated particular regions containing SNP with significantly different effects between the 2 populations by inspecting the consistencies of allele frequency and LD phase between the populations. For

the consistency of allele frequency, average correlations between the 2 populations were 0.862, 0.847, and 0.861 for regions with sizes of 50, 100, and 200 markers, respectively. For consistency of LD phase, average cor-

Table 5. Numbers of significant SNP in the Danish and Chinese Holstein populations, and with significantly different effects between the populations at the chromosome-wide level for milk FA traits

Trait	Number of significant SNP		No. of SNP with different effects between two populations
	Danish Holstein	Chinese Holstein	
C8:0	6	0	0
C10:0	0	8	4
C12:0	0	2	3
C14:0	0	4	0
C14:1	105	318	3
C15:0	0	0	0
C16:0	0	4	0
C16:1	30	9	0
C18:0	1	118	33
C18:1n-9	0	42	2
C18:2n-6	3	16	2
C18:3n-3	4	0	10
CLA911 ¹	2	0	3
C14 index ²	259	413	3
C16 index ³	8	6	1
C18 index ⁴	0	201	1

¹CLA911 = C18:2 *cis*-9,*trans*-11.

²C14 index = $C14:1 / (C14:1 + C14:0) \times 100$.

³C16 index = $C16:1 / (C16:1 + C16:0) \times 100$.

⁴C18 index = $C18:1n-9 / (C18:1n-9 + C18:0) \times 100$.

relations of LD were 0.957, 0.945, and 0.923 for regions with sizes of 50, 100, and 200 markers, respectively (Supplementary Table S6; <http://dx.doi.org/10.3168/jds.2015-9383>). These results indicate that the consistencies of allele frequency and LD phase were high between the 2 populations.

DISCUSSION

Genome-wide association studies enable the identification of novel QTL and candidate genes influencing complex traits. In the present study, we carried out GWAS for 16 milk FA traits in the Chinese and Danish Holstein populations. The GWAS using the data of the joint population (both populations combined) generally detected greater numbers of significant SNP compared with using either population alone because of the larger size of the joint data set. In addition, GWAS based on the joint data detected some SNP with significantly different effects between the 2 populations.

Comparisons to Previous GWAS of Milk FA Traits

Four previous studies have reported the results of GWAS of milk FA traits (Bouwman et al., 2011, 2012; Buitenhuis et al., 2014; Li et al., 2014). Our research was based on data previously used by Li et al. (2014) and Buitenhuis et al. (2014). As accurate measurement of milk FA using GC is expensive and time-consuming, combining 2 sets of genetically related data with consistent LD patterns is an efficient way to extend population size and, hence, increase the detection power for causal mutations that influence milk FA synthesis.

Among the milk FA traits analyzed in current study, 9 FA (C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C14:1, C16:1, and CLA911) were also analyzed in Bouwman et al. (2011) with a winter milk sample and in Bouwman et al. (2012) with a summer milk sample. We found some overlap of results between our study using joint data and the results of Bouwman et al. (2011, 2012). Overlapping regions were on BTA14 (0–26.3 Mbp) for C14:1, C16:0, C16:1, and CLA911, on BTA26 (2.5–41.2 Mbp) for C14:1, on BTA19 (37.3–62.3 Mbp) for C16:0, on BTA20 (8.5–26.1 Mbp) for C18:0, and on BTA26 (1.4–39.0 Mbp) for C14:1.

We found fewer QTL regions associated with milk FA traits than did Bouwman et al. (2011, 2012). The main reason for this discrepancy could be that we defined a QTL region with a more conservative criterion for significant SNP than Bouwman et al. (2011, 2012), even though we used a similar method to define a QTL region. We used a chromosome-wide significance threshold [$-\log_{10}(P\text{-value}) \geq 5.27\text{--}5.77$] to define significant SNP. In contrast, Bouwman et al. (2011)

used a threshold of a $-\log_{10}(P\text{-value})$ of at least 3, and Bouwman et al. (2012) used a threshold of a genome-wide false-discovery rate (FDR) of <0.05 . Our more stringent criterion to define QTL regions was intended to avoid detecting false positives, even if fewer QTL regions were found.

In the Danish population-based analysis, we detected a significant association for C14:1 on BTA26, C16:1 on BTA14, and the C14 index on BTA26, similar to results presented by Buitenhuis et al. (2014). We found a high overlap of the results, with a small difference due to our use of a chromosome-wide significance threshold to define significant SNP compared with the use of an FDR of $P < 0.10$ by Buitenhuis et al. (2014). Comparing our results with those of Li et al. (2014), the advantage of our Chinese population-based analysis was that we used imputed HD information for the GWAS and, consequently, detected greater numbers of significant SNP. Among the milk FA traits, C14:1, C18:1, C14 index, and C18 index were associated with high numbers of significant SNP in the study by Li et al. (2014) and our Chinese population-based analysis.

Compared with the studies by Li et al. (2014) and Buitenhuis et al. (2014), our joint-population analyses (model [2]) detected greater numbers of significant SNP associated with milk FA traits. For example, consider the results obtained for the known genes *DGAT1* and *SCD1*. Significant SNP within or close to *DGAT1* were found to influence 6 FA traits (C14:1, C16:0, C18:1n-9, C18:2n-6, the C14 index, and the C18 index) in the study by Li et al. (2014) and in our Chinese population-based analysis. Likewise, *DGAT1* was found to influence C16:1 in the study by Buitenhuis et al. (2014) and C16:1 and the C16 index in our Danish population-based analysis. When we combined the Holstein populations, we found that significant SNP within or close to *DGAT1* were associated with 10 traits: C14:1, C16:0, C16:1, C18:1n-9, C18:2n-6, C18:3n-3, CLA911, and the C14, C16, and C18 indices. For *SCD1*, 5 traits (C10:0, C14:1, C16:0, and the C14 and C16 indices) were associated with SNP when using the joint data, compared with only C14:1 and the C14 index when the 2 data sets were analyzed separately (Buitenhuis et al., 2014; Li et al., 2014).

GWAS of the Combined Population

Some identified QTL regions were within or close to known genes. Similarly to Li et al. (2014), we identified a chromosome-wide significant QTL region influencing C18:0 that was about 400 kb from *AGPAT3* on BTA1; *AGPAT3* is a member of the acyltransferase family (Cao et al., 2006) and plays a key role in de novo phospholipid biosynthesis (Lu et al., 2005). On BTA14,

the known candidate gene *DGAT1* influenced 10 FA (C14:1, C16:0, C16:1, C18:1n-9, C18:2n-6, C18:3n-3, CLA911, and the C14, C16, and C18 indices). In this case, we identified a greater number of associated traits compared with other studies (Schennink et al., 2009; Stoop et al., 2009; Bouwman et al., 2011, 2012; Buitenhuis et al., 2014; Li et al., 2014).

On BTA20, we found a chromosome-wide significant QTL influencing C14:1 and C18:0. This region was located within *GHR*, a well-known major gene affecting milk yield and composition (Blott et al., 2003). A chromosome-wide significant QTL influencing C18:1n-9 was about 2 Mbp from *PRLR*. The PRLR (prolactin receptor) protein can activate *STAT5A* expression (Jiang et al., 2012) and is associated with milk composition traits (Brym et al., 2005). On BTA23, a chromosome-wide significant QTL that might affect C18:0 was found about 3.5 Mbp from the *BTN1A1* gene; *BTN1A1* (butyrophilin, subfamily 1, member A1) plays an important role in milk lipid droplet formation (Bionaz and Looor, 2008).

We identified a large region (10.00–27.94 Mbp) on BTA26 that affected C14:1 and the C14 index. This region included many reported candidate genes (i.e., *LIPJ*, *LIPK*, *ECHS1*, *SORBS1*, *NFKB2*, *CHUK*, and *SCD1*). When we adjusted the results for the SNP within *SCD1* with the highest $-\log_{10}$ (*P*-value) (BOVINEHD2600005464; 21.14Mbp) in the combined data set analysis, no significant SNP for C14:1 or the C14 index (Figures S3 and S4, respectively; <http://dx.doi.org/10.3168/jds.2015-9383>) were detected at the chromosome-wide significance level. This result indicates that *SCD1* is the most likely candidate gene for this large QTL region on BTA26.

On BTA27, there was a chromosome-wide significant QTL influencing the C14 index, which overlapped with a region influencing C14:0 and C14:1, as reported by Bouwman et al. (2011). This QTL was about 1.2 Mbp from *AGPAT6*, which is a lipid biosynthetic gene required for triacylglycerol production in the mammary epithelium. When *AGPAT6* was knocked out, lactating mice failed to synthesize milk fat (Beigneux et al., 2006).

We identified other QTL regions that are novel or far from known genes. On BTA1, we found a novel genome-wide significant QTL that affected the C18 index. This QTL included *SEMA5B*, which is involved in synapse elimination in hippocampal neurons by influencing n-3 FA (Guo et al., 2011). On BTA5, we detected 2 novel QTL regions that influenced the C18 index. One region (4.27–4.31 Mbp) did not include any known genes, whereas the other region (28.79–29.11 Mbp) included *TFCP2*. DNA methylation status alters

the TFCP2 binding activity at CpG sites, thereby regulating *PPARG1* gene transcription (Baik et al., 2014).

On BTA11, the C18 index was influenced by 2 novel QTL (32.70–32.90 Mbp and 51.89–61.56 Mbp). One QTL region (32.70–32.90 Mbp) was located in the *NRXN1* gene, whereas the other (51.89–61.56 Mbp) included *CTNNA2*. On BTA12, we detected a novel QTL region (69.43–70.26 Mbp) that influenced C18:0 and included the *DCT* gene. On BTA14, other than *DGAT1*, a novel QTL region (65.11–65.45 Mbp) influencing the C18 index was associated with no genes. On BTA15, we identified 2 novel QTL that affected the C18 index: a region in *MAML2* (14.47–14.62 Mbp) and a region in *SORL1* (31.02–32.77 Mbp). On BTA19, a QTL region affecting C15:0 was about 8 Mbp from *SREBF1*, which encodes a transcription factor that regulates the expression of SCD1, which is related to several genes of lipid metabolism (Harvatine and Bauman, 2006). Another QTL region affecting C16:0 was about 8 Mbp from *FASN*; *FASN* (fatty acid synthase) is a multifunctional enzyme with a central role in de novo lipogenesis in mammals (Muñoz et al., 2003).

Comparison of GWAS Results in the Chinese and Danish Holstein Populations

In the analysis of each population separately, we observed a common pattern for the numbers of significant SNP among the traits in both populations. In general, greater numbers of significant SNP were detected in the Chinese compared with the Danish population, and similarities in the detected regions were few. These results are mainly due to the larger size of the Chinese data set, which, therefore, has greater power to detect significant SNP. A high overlap of significant SNP between the 2 populations was found on BTA26 for C14:1 and the C14 index. Overlapping SNP were mainly in a large region (14.93–24.92 Mbp) on BTA26 that covered the known candidate gene *SCD1*. This finding suggests that *SCD1* plays an important role in the regulation of C14:1 and affects the C14 index in both populations. Many significant SNP influenced the C14 index. This finding is most probably due to the similarity of C14:1 with the C14 index. This index has been reported as a good proxy measure of the desaturase activity of C14:1 (Poulsen et al., 2012). Both traits are controlled by genes in common with each other.

Numbers of significant SNP associated with C18:0 and the C18 index differed greatly between the populations. For these traits, fewer significant SNP were found in the joint compared with the Chinese population, and many different regions were detected between the Chinese and Danish populations. The signs of some

SNP effects were opposite in the 2 populations, despite the similar LD patterns between them. When we used model [2], these SNP did not associate with either trait because the average SNP effects were closer to zero in the joint than in the single populations. Therefore, we used model [3] to detect SNP and obtained significant differences in the SNP effect between the 2 populations. For example, there were 32 SNP with significantly different effects for C18:0 between the populations. This phenomenon is consistent with the large differences in the numbers of significant SNP and regions between the single populations and indicates that differences between the Chinese and Danish populations for C18:0 were due to different QTL effects in the 2 populations.

The C18 index had only one SNP (at 71,723,332 bp on BTA 9) with significantly different effects between the 2 populations, even though there were large differences in the numbers of significant SNP and regions for the C18 index between the separate populations. We found 8 SNP with significant differences between the populations at $P < 1.0 \times 10^{-5}$ and 188 SNP at $P < 1.0 \times 10^{-4}$. This result implies that several SNP had relatively large differences in their effects for the C18 index between the 2 populations, even if these SNP effects did not reach the chromosome-wide significance level.

Many SNP that were not significant in either population alone had significantly different effects between the populations. The most likely reason for this phenomenon could be that when the effects of the same SNP were opposite in the 2 populations (i.e., one positive and one negative), the absolute value of the difference between the 2 effects was larger than the absolute value for either SNP effect alone. For example, no significant SNP were found for C10:0 in the Danish population; however, 4 SNP had significantly different effects between the Danish and Chinese populations, because these 4 SNP showed positive effects in the Chinese population and negative effects in the Danish population. Few SNP with significantly different SNP effects were significantly associated with FA in the Chinese population, Danish population, or both. For example, 2 SNP within *FAT4* showed significantly different effects for C18:1n-9 between the 2 populations, as well as significant associations with C18:1n-9 in the Danish population. It may be useful to study the interaction of *FAT4* with environments for milk FA in dairy cattle.

The most likely reason for the significant differences of the SNP effects between the 2 populations for certain FA traits could be genotype by environment interaction. Lillehammer et al. (2009) performed gene by environment interactions for production traits in Australian dairy cattle and found that about two-thirds of detected SNP will cause an increase in environmental sensitivity if used to select for increased production.

Hayes et al. (2009) found that some markers were associated with sensitivity of milk production to feeding level and temperature-humidity index. Those authors mapped the underlying causative mutations for this sensitivity to a small genomic interval containing 2 promising candidate genes. Although phenotypes were obtained from GC analyses for both populations, the measure of milk FA traits may be not exactly the same between laboratories. The power to detect QTL regions and differences in SNP effects between the 2 populations might be reduced due to differences in phenotype measurements and sample sizes (Rosenberg et al., 2010; Ball, 2013).

CONCLUSIONS

Our study showed that combining data from different populations allowed detection of a larger number of significant SNP compared with analysis based on a single population alone. The genome-wide association study (model [2]) identified 830 genome-wide and 1,593 suggestive significant SNP associated with 16 milk FA traits. Some of these SNP were located within or near to previously reported genes and QTL regions, whereas some of the SNP were novel. Sixty-five significant SNP across all traits had significantly different effects in the 2 populations. These findings are helpful for follow-up studies to identify causal mutations and to assess their interaction with environments for milk FA in dairy cattle.

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