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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; Schennink 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 ge-

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netic 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 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 × 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 ± 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:

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