# Effects of A17924G Genotypes Associated with Thioesterase Domain of Fatty Acid Synthase and K232A Genotypes of Diacylglycerol Acyltransferase-1 on Milk Fatty Acid Composition in Holstein Dairy Cows

## A.S. Leaflet R2433

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### Summary and Implications

The objective of this study was to determine if variations in single nucleotide polymorphism (SNP) in thioesterase domain of the fatty acid synthase (g.17924 A>G Threonine>Alanine) and in diacylglycerol acyltransferase-1 (g.10433/10434 GC/AA Alanine>Lysine) genes would explain variations in milk fatty acid composition among Holstein dairy cattle. About 200 cows participated in the study. Milk samples were collected monthly throughout the first ten months of lactation and analyzed for milk fatty acid composition by gas chromatography. Blood samples were used to obtain a DNA sample for each animal. Milk from cows with g.17924GG genotype had lower atherogenic index [AI; (12:0 + 4(14:0) + 16:0)/(MUFA + PUFA)] compared with milk from cows of g.17924AG genotype (P=0.007). Likewise, milk from cows with p.232AA genotype had lower AI compared with that from cows of p.232KK genotype (P<0.016). The decrease in AI for cows with g.17924GG and p.232AA genotypes was achieved by the decrease in the concentration of palmitic acid (P=0.06 and P<0.0001, respectively) and by the increase in the concentration of mono- and poly-unsaturated fatty acids in milk for both genotypes. The results of this study indicate the potential of using earlier mentioned SNPs as DNA markers to select breeding animals that produce progeny with a healthier milk fatty acid composition.

### Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States. Elevated blood cholesterol and low-density lipoprotein-cholesterol are the main factors contributing to the CVD development in humans. The concentration of those metabolites in blood is increased when foods rich in saturated fatty acids (SFA) are consumed. The major sources of those fatty acids are animal products that provide collectively 56% of total lipids, 74% of SFA, and 100% of cholesterol consumed by humans. Decreasing the percentage of SFA in milk and other animal products may lead to the improvement of the healthfulness of those foods and decrease in the incidence of CVD in humans.

Contributions of individual fatty acids to atherogenic potential for a lipid source or a diet are summarized by an atherogenic index (AI) that was developed by Ulbricht and Southgate in 1991. The AI is calculated as a ratio of the sum of concentrations of lauric (12:0), four times myristic (14:0), and palmitic (16:0) acids to the sum of concentrations of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

The focus of this study is two genes from the milk fat biosynthetic pathway: thioesterase (TE) domain of fatty acid synthase (FAS) and diacylglycerol acyltransferase-1 (DGAT1). The TE domain of FAS is responsible for terminating the pathway of fatty acid biosynthesis by releasing the newly synthesized fatty acid. The TE domain, therefore, is essential in determining the chain length of FAS products and, consequently, milk fatty acid composition. The single nucleotide polymorphism (SNP) in TE domain of FAS gene (g.17924 A>G Threonine>Alanine) was shown to be associated with beef fatty acid composition (Zhang et al., 2007). The DGAT1 catalyzes the rate-limiting step in triacylglycerol biosynthesis by selectively attaching fatty acids to the sn-3 position on the glycerol backbone of triacylglycerol molecules. The A232K polymorphism in DGAT1 gene was shown to be associated with milk fat yield.

### Hypothesis

Our hypothesis is that the genetic polymorphisms in TE domain of FAS and in DGAT1 can be associated with milk fatty acid composition. As a result, animal breeders may select for cows that will produce milk with greater healthfulness (lower AI or SFA/unsaturated fatty acids (UFA)).

### Objectives

- Determine milk fatty acid composition from Holstein dairy cows over entire lactation period.
- Determine if there are associations between milk fatty acid composition and genetic polymorphisms in TE domain of FAS and in DGAT1.

## Materials and Methods

### Milk fatty acid composition

About 200 multiparous Holstein dairy cows participated in the study. Milk samples were collected monthly throughout the lactation. Milk production, milk fat, sire line, and lactation number were recorded for each animal and used in statistical analysis of the data. The fatty acid composition of milk was determined by gas chromatography after milk total lipid extraction.

### Genotyping

Genotyping of A17924G and A232K SNPs was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with agagctgacggactccacac and tgggctccgtgctggccctgatggtcta as forward and gcctgatgcactcgatgtag and ttgagctcgtagcacagggtgggggcga as reverse primers, respectively. The PCR products for A17924G and A232K SNPs were digested with MscI and EaeI restriction enzymes, respectively.

### Statistical analysis

The PROC MIXED procedure of SAS with month of lactation as a repeated statement was used to analyze the data. The mixed model included genotype as a fixed effect, sire line as a random effect, and milk production, percentage of milk fat, and lactation number as covariates. Autoregressive heterogeneous variance-covariance structure was used in the analysis and Tukey-Kramer adjustment was applied to account for multiple comparisons between different genotypes. The data are presented as least square means (±SE).

## Results and Discussion

The distribution of genotypes along with the number of animals for two SNPs in TE domain of FAS and DGAT1 is shown in table 1.

### Effects of A17924G genotypes associated with TE domain of FAS on AI and milk fatty acid composition.

The cattle with GG genotype have lower AI and lower percentage of SFA, lower ratio of SFA/UFA, but higher percentages of UFA, MUFA, and PUFA than do the cattle with AG genotype (table 2). In particular, the cattle with GG genotype have higher percentages of C18:1, C18:2 and C10:0 than do the cattle with AG genotype. The fact that cows with GG genotype have higher percentages of C18:1 and C18:2 and tend to have lower percentage of C16:0 (P=0.06) compared with the cows with AG genotype can be explained by GG genotype being in a linkage disequilibrium with SNPs of fatty acid elongase, resulting in the phenotype with higher rates of C16:0 and C16:1 elongation. The cattle with GG genotype also have lower percentage of C14:0 than do the cattle with AA genotype. This difference, however, does not contribute significantly to the difference in AI between GG and AA genotypes.

### Effects of K232A genotypes of DGAT1 on AI and milk fatty acid composition

The cattle with AA genotype have lower AI, lower percentage of SFA, and lower SFA/UFA ratio, but higher percentages of UFA, MUFA, and PUFA than do the cattle with KK genotype. The cattle with AA genotype also have lower AI and lower percentage of SFA than do the cattle with KA genotype. In particular, the cattle with AA genotype have lower percentages of C14:1, C15:0, C16:0, and C16:1, but higher percentages of C18:0 and C18:1 than do the cattle with KK and KA genotypes. The cattle with AA genotype also have lower percentage of C14:1 than do the cattle with KK genotype and lower percentage of C10:0 and C12:0 than do the cattle with KA genotype. Finally, the cattle with AA genotype have higher percentage of C18:2 than do the cattle with KK genotype. The fact that cows with AA genotype have higher percentages of C18:0, C18:1, and C18:2 fatty acids in their milk than do cows with KK and KA genotypes can be explained by higher substrate specificity of DGAT1 in the cows with AA genotype for 18 carbon fatty acids compared with saturated and unsaturated medium chain and 16 carbon fatty acids that are higher in milk of the cows with KK and KA genotypes.

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**Table 1. Genotype distribution of SNPs in TE domain of FAS and DGAT1.**

| Variable | SNP | | | | | |
|---|---|---|---|---|---|---|
| | TE domain of FAS g. 17924 A>G (T >A) | | | DGAT1 g. 10433/10434 GC/AA (A>K) | | |
| | AA | GG | AG | KK | AA | KA |
| Number of animals | 23 | 69 | 97 | 9 | 130 | 57 |
| Genotype frequency, % | 12.2 | 36.5 | 51.3 | 4.6 | 66.3 | 29.1 |

**Table 2. Effects of A17924G genotypes on fatty acid composition in milk.**

| Trait | TE domain of FAS g. 17924 A>G (T >A) | | |
|---|---|---|---|
| | AA | GG | AG |
| AI | 2.12 ± 0.05[a, b] | 2.12 ± 0.03[b] | 2.23 ± 0.03[a] |
| SFA, wt % | 64.24 ± 0.47[a, b] | 64.44 ± 0.32[b] | 65.36 ± 0.29[a] |
| UFA, wt % | 35.76 ± 0.47[a, b] | 35.56 ± 0.32[b] | 34.64 ± 0.29[a] |
| MUFA, wt % | 30.89 ± 0.43[a, b] | 30.91 ± 0.28[a] | 30.18 ± 0.26[b] |
| PUFA, wt % | 4.76 ± 0.08[a] | 4.58 ± 0.06[a] | 4.43 ± 0.05[b] |
| SFA/UFA | 1.85 ± 0.04[a] | 1.84 ± 0.03[a, b] | 1.94 ± 0.02[a, c] |
| 10:0, wt % | 2.46 ± 0.06[a] | 2.46 ± 0.04[a, b] | 2.55 ± 0.04[a, c] |
| 12:0, wt % | 3.03 ± 0.13 | 2.89 ± 0.08 | 2.96 ± 0.07 |
| 14:0, wt % | 10.98 ± 0.25[a,c] | 10.24 ± 0.15[b,d] | 10.44 ± 0.12[c,d] |
| 14:1, wt % | 0.64 ± 0.05 | 0.64 ± 0.03 | 0.68 ± 0.03 |
| 15:0, wt % | 1.05 ± 0.06[a,b] | 0.98 ± 0.03[a] | 1.07 ± 0.03[b] |
| 16:0, wt % | 29.10 ± 0.29[a] | 29.53 ± 0.19[a, c] | 29.97 ± 0.17[b, c] |
| 16:1, wt % | 1.67 ± 0.10 | 1.73 ± 0.06 | 1.77 ± 0.05 |
| 18:0, wt % | 11.99 ± 0.44 | 11.66 ± 0.26 | 11.77 ± 0.23 |
| 18:1, wt % | 22.60 ± 0.35[a] | 22.75 ± 0.23[a, b] | 21.93 ± 0.20[a, c] |
| 18:2, wt % | 3.12 ± 0.05[a] | 2.95 ± 0.04[b] | 2.84 ± 0.03[c] |

[abcd] Means with different superscripts differ (P<0.05)

**Table 3. Effects of K232A genotypes on fatty acid composition in milk.**

| Trait | DGAT1 g. 10433/10434 GC/AA (A>K) | | |
|---|---|---|---|
| | KK | AA | KA |
| AI | 2.33 ± 0.07[a] | 2.15 ± 0.03[b] | 2.24 ± 0.03[a] |
| SFA, wt % | 66.51± 0.61[a] | 64.69 ± 0.26[b] | 65.37 ± 0.33[a] |
| UFA, wt % | 33.49 ± 0.61[a] | 35.31 ± 0.26[b] | 34.63 ± 0.33[a, b] |
| MUFA, wt % | 29.36 ± 0.55[a] | 30.68 ± 0.24[b, c] | 30.14 ± 0.30[a, c] |
| PUFA, wt % | 4.10 ± 0.11[a] | 4.55 ± 0.05[b] | 4.51 ± 0.06[b] |
| SFA/UFA | 2.01 ± 0.05[a] | 1.88 ± 0.02[b] | 1.93 ± 0.03[a, b] |
| 10:0, wt % | 2.53 ± 0.07[a] | 2.47 ± 0.04[a, b] | 2.60 ± 0.04[a, c] |
| 12:0, wt % | 2.93 ± 0.08[a] | 2.89 ± 0.04[a, b] | 3.02 ± 0.05[a, c] |
| 14:0, wt % | 10.59 ± 0.31 | 10.46 ± 0.12 | 10.33 ± 0.16 |
| 14:1, wt % | 0.85 ± 0.04[a] | 0.72 ± 0.02[b] | 0.82 ± 0.02[a] |
| 15:0, wt % | 1.12 ± 0.03[a] | 0.95 ± 0.01[b] | 1.06 ± 0.02[a] |
| 16:0, wt % | 31.63 ± 0.37[a] | 29.37 ± 0.15[b] | 30.17 ± 0.20[c] |
| 16:1, wt % | 1.96 ± 0.05[a] | 1.70 ± 0.03[b] | 1.88 ± 0.03[a] |
| 18:0, wt % | 11.20 ± 0.29[a] | 11.86 ± 0.14[b] | 11.38 ± 0.17[a] |
| 18:1, wt % | 21.15 ± 0.46[a] | 22.62 ± 0.18[b] | 21.60 ± 0.23[a] |
| 18:2, wt % | 2.61 ± 0.07[a] | 2.94 ± 0.03[b] | 2.89 ± 0.04[b] |

[abcd] Means with different superscripts differ (P<0.05)