Association of the *OLR1* Gene with Milk Composition in Holstein Dairy Cattle

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ABSTRACT

Oxidized low-density lipoprotein receptor (OLR1) is the major protein that binds, internalizes, and degrades oxidized low-density lipoprotein. The role of OLR1 in lipid metabolism and the results of previous whole-genome scan studies prompted the investigation of *OLR1* as a candidate gene affecting milk composition traits. Direct cDNA and genomic sequencing of *OLR1* revealed 2 single nucleotide polymorphisms (SNP) in exon 4, 5 SNP in intron 4, and 1 in the 3' untranslated region (UTR). Four intragenic haplotypes comprising SNP positions 7,160, 7,161, 7,278, 7,381, 7,409, 7,438, 7,512, and 8,232 were inferred. Haplotype analysis showed that one of the haplotypes was associated with a significant increase in fat yield and fat percentage. Single SNP analysis showed that allele C of SNP 8,232 (in the 3'-UTR) had significant effects on fat yield and fat percentage, whereas SNP 7,160 and 7,161 (in exon 4) had no significant effects. Both single SNP and haplotype analyses indicate that SNP 8,232 in the 3'-UTR is associated with milk fat yield and percentage and it may be in linkage disequilibrium with the functional polymorphism. To provide support for the hypothesis that SNP 8,232 is responsible for *OLR1* expression, *OLR1* expression levels in individuals bearing different genotypes were assessed. It was found that *OLR1* expression was reduced in genotype AA individuals compared with CC and AC individuals, suggesting that A at position 8,232 may be the nucleotide causing decreased *OLR1* expression. The 3'-UTR polymorphism found in this study might control translation or stability of *OLR1* mRNA.

Key words: candidate gene, milk composition, oxidized low-density lipoprotein receptor

INTRODUCTION

The oxidized form of the low-density lipoprotein (oxLDL) is involved in endothelial cell injury, dysfunc-

tion, and activation, all of which are implicated in the development of atherosclerosis (Mehta and Li, 1998). It has been shown that oxLDL and its lipid constituents have numerous damaging effects on secretory activities of the endothelium, including induction of apoptosis (Imanishi et al., 2002). The major protein that binds, internalizes, and degrades oxLDL, oxidized LDL receptor 1 (**OLR1**), was initially identified in bovine aortic endothelial cells by Sawamura et al. (1997). In addition to binding oxLDL, OLR1 removes aged and apoptotic cells from blood circulation (Oka et al., 1998).

The bovine *OLR1* gene encodes 270 AA and has 72% identity to the human protein (Sawamura et al., 1997). Aoyama et al. (1999) determined the structure of human *OLR1*, and found 6 exons, of which the first 3 corresponded to the N-terminal cytoplasmic, transmembrane, and connecting neck domains, and the last 3 encoded the lectin domain. The genomic sequence of bovine *OLR1*, recently released by Baylor College of Medicine, contains 5 exons (GenBank accession no. NW_215807).

Several QTL affecting milk production traits have been reported on bovine chromosome 5 near *OLR1* (Khatkar et al., 2004). Heyen et al. (1999) reported a putative QTL affecting fat percentage at a position 100 cM from the centromere in large granddaughter- and daughter-design populations composed of 1,068 sons and 3,264 daughters, respectively. Olsen et al. (2002) reported a QTL affecting fat yield at a position 115 cM from the centromere. Also, Ashwell et al. (2004) reported that a QTL located at 87 cM was associated with fat percentage in a North American Holstein population. Likewise, QTL for other milk production traits in the *OLR1* region have also been identified. For example, De Koning et al. (2001) reported that a QTL at 107 cM was associated with milk yield in the Finnish Ayrshire population. Using the same population, Viitala et al. (2003) reported another QTL at 98 cM that was associated with milk yield. A QTL for milk yield was also reported at position 109 cM by Bennewitz et al. (2003). Rodriguez-Zas et al. (2002) reported that a QTL affecting protein yield was located at position 91 cM.

Based on the aforementioned studies on the role of human OLR1 in lipid metabolism, including degrada-

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Table 1. Primer sequences used in amplification of cDNA and genomic fragments

Primer ¹ (location)	Sequence	Product type	Length (bp)
OLR5 (exon 1)	ATGACTGTTGATGACCCCAAG	cDNA	812
OLR6 (exon 5)	CACTGTGCTCTCAATAGATTCGCCT		
OLR7 (exon 4)	CTCCTTTGACGCCCACTT	DNA	670
OLR8 (exon 5)	GGTCCCTGAAGGATACATAC		
OLR3 (exon 5)	AAGGCGAATCTATTGAGAGC	DNA	270
OLR4 (3'-untranslated region)	acttctctgaagtctgca		
OLR9 (exon 4)	GGAATTCATCCAGCAAATGATTGCC	cDNA	89
OLR10 (exon 5)	TTGACAACCCCATCCAGAAG	(real-time PCR)	

¹OLR = Oxidized low-density lipoprotein receptor.

tion of oxLDL, and on previous QTL studies, *OLR1* was chosen as a candidate gene for association tests with milk production traits in dairy cattle. In this study several lines of evidence are presented for a single nucleotide polymorphism (SNP) in the 3' untranslated region (UTR) that is significantly associated with fat yield and fat percentage in milk.

MATERIALS AND METHODS

Populations and Phenotypic Data

Semen samples from 29 Holstein sires and their 1,433 sons were obtained from the Cooperative Dairy DNA Repository (CDDR), which is maintained by the USDA Bovine Functional Genomics Laboratory (Beltsville, MD). Daughter yield deviation (DYD) data for milk yield, milk protein and fat yields, milk protein and fat percentages, productive life, and SCS were obtained from the USDA Animal Improvement Programs Laboratory (Beltsville, MD). Samples of DNA from 19 Brown Swiss, 15 Guernsey, 13 Jersey, 12 *Bos indicus*, 18 *Bison bison*, and 3 Gaur individuals were included in this study for allele frequency comparisons.

Detection of SNP

Several SNP were detected in the coding regions of the *OLR1* gene by direct sequencing of reverse transcription PCR (RT-PCR) products obtained from a pool of cDNA from 50 animals. Total RNA was extracted from various bovine tissues (adult and fetus, obtained from an abattoir), pooled, and used for RT-PCR amplification as previously described (Khatib et al., 2005). The primers OLR5 and OLR6 (Table 1) were designed to amplify the total cDNA sequence of the gene. The SNP in the genomic sequence were detected by direct sequencing of pooled genomic DNA samples. The DNA

pools were constructed from 220 bovine samples and amplified with unlabeled primers. Primers OLR7 and OLR8 (Table 1) were used to amplify genomic DNA sequence of intron 4. Primers OLR3 and OLR4 (Table 1) were used to amplify 270 bp of genomic DNA sequence in the 3'-UTR. The PCR and RT-PCR products were sequenced and SNP were identified by visually inspecting sequence traces.

DNA Genotyping and Haplotype Construction

Genomic DNA was extracted from semen samples by standard methods using proteinase K and phenol-chloroform. Twenty-nine sires and their 1,433 sons were genotyped in this study. For the polymorphism at position 8,232 (A/C) of *OLR1* (GenBank accession no. NW_215807), all sons were genotyped using the restriction enzyme *Pst*I. The digestion products were separated on a 3.0% agarose gel. The A allele (uncut) was indicated by a band at 270 bp and the C allele (cut) resulted in a band at 250 bp. For the polymorphisms at positions 7,160 and 7,161, individuals were genotyped by direct sequencing. For polymorphisms in intron 4 at positions 7,278, 7,381, 7,409, 7,438, and 7,512, all 29 sires as well as 4 to 6 heterozygous and 4 to 6 homozygous sons within each sire family were genotyped by direct sequencing using primers OLR7 and OLR8 (Table 1). Intragenic haplotypes were inferred based on the genotypes of sires and their sons. For example, for a sire having the genotypes C/T, A/G, T/G, A/G, C/T, C/G, C/G, and A/C, haplotypes C-A-T-A-C-C-C-A and T-G-G-G-T-G-G-C were inferred based on one son being homozygous for the haplotype C-A-T-A-C-C-C-A and the other son being homozygous for the haplotype T-G-G-G-T-G-G-C. For individuals with homozygous genotypes, for example, C/C, A/A, T/T, A/A, C/C, C/C, C/C, A/A, the haplotype C-A-T-A-C-C-C-A was inferred.

Statistical Analyses

Maternal allele frequencies of *OLR1* were estimated following Thaller et al. (2003), in which all sons from homozygous sires and all homozygous sons of heterozygous sires at position 8,232 were used. The allele frequencies were estimated using the formula

$$P_c = \frac{n_{CC} + n'_{AC} + n''_{CC}}{n_{CC} + n_{AA} + n'_{AC} + n'_{AA} + n''_{AC} + n''_{CC}}$$

where n_{CC} and n_{AA} are the number of homozygous CC and AA sons within heterozygous sires; n'_{AC} and n'_{AA} are the number of heterozygous AC and homozygous AA sons, respectively, from homozygous AA sires; and n''_{AC} and n''_{CC} are the numbers of AC and CC sons, respectively, of CC sires.

The data were analyzed by an allele substitution model using the PROC GLM function of SAS (SAS Institute, 1999). For single SNP analysis, the model was as follows:

$$y_{ij} = \mu + \text{sire}_i + bx_{ij} + e_{ij} \quad [1]$$

and for analysis of gene haplotypes, the model was

$$Y_{ij} = \mu + \text{sire}_i + \sum_{k=1}^{M-1} \beta_k A_{ijk} + e_{ij} \quad [2]$$

where Y_{ij} is the DYD of the trait for son j of sire i ; μ is the mean; sire_i is the fixed effect of sire i ; and e_{ij} is the residual. For model [1], b is the regression coefficient representing half of the gene substitution effect (Falconer and Mackay, 1996) and x_{ij} is the number of alleles (0, 1, or 2). For model [2], $A_{ijk} = 0, 1, 2$, is the number of copies of haplotype k present in the ij th individual, where A_0 represents the most frequent of M haplotypes, and the remaining haplotypes are denoted $A_1, \dots, A_k, \dots, A_{(M-1)}$; β_k are partial regression coefficients corresponding to the effect of haplotype k as a deviation from the effect of the most frequent haplotype (A_0), which is set to zero to make the model have full rank (Batra et al., 1989; Weigel et al., 1990; Sharif et al., 1999). Reliability of the son's DYD was incorporated into the models to obtain weighted least squares estimates for the allele effects.

Models [1] and [2] were fitted using weighted least squares, with weights based on reliability (Israel and Weller, 1998). For model [2], significance of associations was determined for each trait separately by an F -test on the sum of squares explained by the combined effect of haplotypes. Then, for traits with significant associations, estimates of the effect of individual haplotypes,

as a deviation from the effect of the most frequent haplotype, were evaluated for significance.

Real-Time PCR

Expression levels of *OLR1* were evaluated by real-time PCR (Bieche et al., 2000; Karsai et al., 2002) using a standard curve method (Robert et al., 2002). Thirty-three RNA samples obtained from heart tissues (from an abattoir) were extracted using the Qiagen RNeasy kit (Qiagen, Valencia, CA). The primers used for real-time PCR reactions were OLR9 and OLR10 (Table 1). The RNA was reverse transcribed using the iScript cDNA Synthesis kit (BioRad, Hercules, CA). The cDNA synthesis reaction (20 μ L) included 4 μ L of 5 $\times$ iScript reaction mix, 1 μ L of iScript reverse transcriptase, and 2 μ L of RNA. The reaction conditions were performed as recommended by the manufacturer. Expression levels of *OLR1* and the housekeeping gene, acidic ribosomal phosphoprotein (*PO*), were evaluated by real-time PCR using a DNA Engine—Opticon 2 Detection System (MJ Research, Watertown, MA). The PCR products were detected with SYBR Green I using iQ SYBR Green Supermix kit (BioRad). Each PCR reaction mix (25 μ L) contained 12.5 μ L of Supermix (contains dNTP, Taq DNA polymerase, SYBR Green I, and fluorescein), 250 nM of forward primer, 250 nM of reverse primer, and 1.6 μ L of RT-PCR products. Thermal cycling conditions were 95°C for 10 min, followed by 40 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s, and finally 72°C for 5 min. Melting curve analysis and agarose gel electrophoresis were performed after real-time PCR reaction to monitor PCR specificity.

The threshold cycle (C_t) numbers were determined for the amplified cDNA of the bovine *OLR1* mRNA and for the housekeeping gene in each sample during real-time PCR. Standard curves for PCR amplification of cDNAs of *OLR1* and *PO* genes were constructed using purified PCR products of the mRNA of the 2 genes and 5 serial dilutions of the products. Standard curves were generated by plotting the C_t values (y-axis) against the logarithm of input purified PCR products (x-axis). For each sample, the amounts of *OLR1* and *PO* were determined from the standard curve. Then, the *OLR1* amount was divided by the amount of *PO* to obtain a normalized *OLR1* value, expressed as the ratio of *OLR1* to *PO*. The PCR reactions were repeated for samples showing amplification efficiencies lower than 80%.

RESULTS

The exact location of bovine *OLR1* is not known, but based on combined data from different cattle maps we estimated the location of the gene to be in the interval

Table 2. Intragenic haplotypes at the *OLR1*¹ gene and their frequencies

Haplotype	Sequence ²	Frequency ³
1	C-A-T-A-C-C-C-A	0.725
2	C-A-T-C-C-C-C-C	0.170
3	C-G-G-G-C-G-G-C	0.052
4	T-G-G-G-T-G-G-C	0.053

¹OLR = Oxidized low-density lipoprotein receptor.

²Polymorphic site at positions 7,160, 7,161, 7,278, 7,381, 7,409, 7,438, 7,512, and 8,232.

³Haplotype frequencies were estimated based on the maternal alleles of the sons.

of 106 to 108 cM of chromosome 5. In the cattle second-generation radiation map, *OLR1* was mapped between the markers BMS1658 and BMS772 (<http://www.genome.iastate.edu>). These 2 markers were mapped in the interval of 106 to 108 cM in the USDA Meat Animal Research Center map (<http://www.marc.usda.gov>).

Estimation of Allele and Haplotype Frequencies in Cattle Breeds

Direct sequencing of pooled RT-PCR products for the total coding sequence of *OLR1* revealed 2 SNP in exon 4 at positions 7,160 (C/T) and 7,161 (A/G). The SNP 7,160 is a missense mutation in which Thr is replaced by Met, and SNP 7,161 is a synonymous substitution. Direct sequencing of genomic DNA at intron 4 revealed 5 SNP at positions 7,278, 7,381, 7,409, 7,438, and 7,512. Direct sequencing of the 3'-UTR (genomic DNA) of *OLR1* revealed 1 SNP (A/C) at position 8,232. Four intragenic haplotypes (Table 2) comprising positions 7,160, 7,161, 7,278, 7,381, 7,409, 7,438, 7,512, and 8,232 were inferred in a sample of 633 individuals from the CDDR resource population. Haplotype 1 was inferred by genotyping individuals at position 8,232; haplotype 3 was inferred by genotyping individuals at positions 7,160 and 7,161; and haplotypes 2 and 4 were inferred by genotyping at positions 8,232 and 7,161, respectively. The number of individuals carrying haplotypes 1, 2, 3, and 4 were 459, 107, 33, and 34, respectively. Thus, for construction of haplotypes, it was unnecessary to genotype SNP 7,278, 7,381, 7,409, and 7,512 in intron 4. These SNP were in a conserved haplotype block along with SNP 7,160, 7,161, and 8,232.

For the CDDR Holstein population, 7 sires at SNP 8,232 were homozygous CC, 8 sires were homozygous AA, and 14 sires were heterozygous. The maternal C allele frequency at SNP 8,232, estimated in 1,433 Holstein sons, was 0.54 (standard error = 0.015). The C allele frequency was 0.87 in Guernsey and 0.83 in *Bos indicus* whereas its frequency in *Bison bison*, Brown Swiss, Jersey, and Gaur was 1.0.

Table 3. Estimates of the allele substitution effects (of allele C at SNP 8,232) and SE for production and functional traits in the CDDR¹ population

Trait	$\alpha/2$ (SE)	P-value
Milk yield, kg	11.83 $\pm$ 29.24	0.6858
Fat yield, kg	3.72 $\pm$ 1.07	0.0005
Fat percentage	0.014 $\pm$ 0.005	0.0033
Protein yield, kg	0.56 $\pm$ 0.73	0.4391
Protein percentage	0.001 $\pm$ 0.002	0.6588
Productive life, mo	0.127 $\pm$ 0.105	0.2255
SCS	0.006 $\pm$ 0.010	0.5407

¹CDDR = Cooperative Dairy DNA Repository.

Effects of Single SNP on Production Traits

To study the effects of *OLR1* variants on production and functional traits, a single SNP analysis was performed only for SNP at positions 7,160, 7,161, and 8,232. Analysis of SNP 7,160 and 7,161 did not show any significant effects on any of the examined traits (data not shown). In contrast, analysis of SNP 8,232 revealed significant effects on both fat yield and fat percentage. Table 3 shows estimates of the allele substitution effects for production and functional traits in 1,433 individuals from the CDDR population. Allele C was associated with significant increases in fat yield ($P = 0.0005$) and fat percentage ($P = 0.0033$). It is worth noting that allele C did not show any significant unfavorable effects on the other examined traits.

Effects of the *OLR1* Haplotypes on Production Traits in the CDDR Holstein Population

An allele substitution model, in which haplotype 1 was set as a reference haplotype, was used to estimate the effects of *OLR1* haplotypes on milk production and health traits in the Holstein population. Table 4 shows the analysis of the effects of the *OLR1* region, which combines information of all haplotypes. The *OLR1* region as a whole showed a significant effect on fat percentage ($P = 0.0265$) and fat yield ($P = 0.0224$). In contrast, the *OLR1* region did not show significant effects

Table 4. Significance of the effects of the *OLR1*¹ gene region on production and health traits

Trait	F-test	P-value
Milk yield, kg	0.39	0.7580
Fat yield, kg	3.22	0.0224
Fat percentage	3.09	0.0265
Protein yield, kg	0.62	0.6010
Protein percentage	0.15	0.9320
Productive life, mo	1.08	0.3570
SCS	0.56	0.6410

¹OLR = Oxidized low-density lipoprotein receptor.

Table 5. Estimates of the substitution effects of the *OLR1*¹ haplotypes for milk production and health traits as a deviation from the effect of the most frequent haplotype (haplotype 1)

Trait	Haplotype 2	Haplotype 3	Haplotype 4
Milk yield, kg	10.6 ± 52.8	22.0 ± 89.6	-74.6 ± 77.5
Fat yield, kg	5.81 ± 1.89*	-1.52 ± 3.21	0.96 ± 2.78
Fat percentage	0.022 ± 0.008**	-0.009 ± 0.014	0.015 ± 0.012
Protein yield, kg	0.73 ± 1.31	0.36 ± 2.22	-2.19 ± 1.92
Protein percentage	0.0024 ± 0.0037	-0.0007 ± 0.0063	0.0002 ± 0.0055
Productive life, mo	-0.008 ± 0.179	0.505 ± 0.316	0.319 ± 0.281
SCS	-0.009 ± 0.019	-0.024 ± 0.032	0.021 ± 0.028

¹OLR = Oxidized low-density lipoprotein receptor.**P* = 0.0022; ***P* = 0.0064.

on milk yield, protein yield and percentage, productive life, or SCS. Table 5 shows the estimates of the substitution effects of the *OLR1* haplotypes for milk production and health traits as a deviation from the effect of haplotype 1. Haplotype 2 was associated with a significant increase in fat yield (*P* = 0.0022) and in fat percentage (*P* = 0.0064). Haplotypes 3 and 4 did not show significant effects on any of the examined traits.

The detected effects of haplotype 2, which has C at position 8,232, motivated us to estimate the substitution effects of haplotypes 3 and 4 for fat yield and fat percentage as deviations from the effect of haplotype 2. These two haplotypes also have C at position 8,232; hence, significant differences between haplotypes 3 and 4 and haplotype 2 would indicate the presence of additional SNP on haplotype 2 affecting fat yield and fat percentage. For fat yield, haplotypes 3 and 4 did not show significant differences compared with haplotype 2; for fat percentage, haplotype 3 showed a suggestive difference (*P* = 0.0854) compared with haplotype 2.

Expression Analysis of *OLR1* Transcripts in Heart Tissues

To test the expression level of *OLR1* in individuals with the C allele compared with individuals with the A allele at SNP 8,232, DNA samples from 33 individuals were first genotyped using PCR-RFLP and direct genomic sequencing. Twenty individuals were homozygous CC, 9 individuals were heterozygous AC, and 4 individuals were homozygous AA. Then, expression levels of *OLR1* in hearts from these individuals were evaluated using real-time quantitative PCR. The level of expression of *OLR1* transcripts in relation to *PO* transcripts was considerably lower in individuals bearing genotype AA (*OLR1/PO* ratio = 35.4) compared with CC individuals (*OLR1/PO* ratio = 170.5). The *OLR1/PO* ratio in heterozygous individuals was 77.4. Thus, C at position 8,232 in the 3'-UTR may be the functional nucleotide that increases *OLR1* expression, and it is in partial linkage disequilibrium with the functional polymorphic site in *OLR1* or in some other closely linked gene.

DISCUSSION

In this study, we report the effect of nucleotide C at position 8,232 in the 3'-UTR of *OLR1*, on milk fat yield and milk fat percentage in dairy cattle. Positional comparative candidate gene analysis and previous QTL linkage mapping results were used to select *OLR1* as a candidate gene affecting milk composition traits. To search for polymorphic sites in the gene, pools of cDNA extracted from a wide range of cattle tissues and pools of genomic DNA extracted from semen samples were sequenced. Using the pooled sequencing approach, 2 SNP in exon 4, 5 SNP in intron 4, and 1 SNP at position 8,232 in the 3'-UTR were identified. The SNP 7,160 (C/T) is a missense mutation in which Thr is replaced by Met, and SNP 7,161 (A/G) is a synonymous mutation.

The first 3 exons of the human *OLR1* correspond to the cytoplasmic domain, the transmembrane domain, and the neck domain, whereas exons 4 through 6 encode the lectin-like domain (Aoyama et al., 1999). Chen et al. (2001) conducted series of targeted mutations in the lectin-like domain to identify structures required for oxLDL binding. They found that the lectin-like domain is essential for binding and endocytosis of oxLDL. To test whether the bovine Thr-Met SNP 7,160 found in exon 4 that encodes the lectin-like domain is associated with milk yield and composition traits, we genotyped the 29 Holstein sires of the CDDR population. Genotyping revealed 5 sires heterozygous for SNP 7,160, 8 sires heterozygous for SNP 7,161, and 14 sires heterozygous for SNP 8,232. All sons of the 5 sires heterozygous for SNP 7,160 were genotyped by direct sequencing for both SNP 7,160 and 7,161. In the CDDR population, 4 intragenic haplotypes were identified and tested for association with milk production traits. Only haplotype 2 was associated with a significant increase in fat yield and fat percentage. Thus, it was concluded that the amino acid substitution at position 7,160 (included in haplotype 4) was not responsible for the effects of *OLR1* on fat yield and fat percentage.

The results of the single SNP analysis showed that allele C of SNP 8,232 had significant effects on fat yield

and percentage, whereas SNP 7,160 and 7,161 had no significant effects. In the CDDR population, the correlation between milk yield and fat yield was 0.59 and between protein yield and fat yield it was 0.69 (Khatib et al., 2005). However, SNP 8,232 showed significant effects only on fat yield and fat percentage and not on milk and protein yields, which suggested a specific role of *OLR1* in fat metabolism in tissues other than the mammary gland.

The results show that the effects of haplotype 2 were not significantly different from the effects of haplotypes 3 and 4. Because haplotypes 2, 3, and 4 all included allele C at SNP 8,232, the results of single SNP analysis and haplotype analysis strongly indicate that SNP 8,232 in the 3'-UTR might be linked to milk fat yield and percentage. To search for other SNP in the 3'-UTR, 790 bp of genomic DNA from all 29 sires was sequenced, but no additional SNP were identified.

To provide support for the hypothesis that SNP 8,232 is responsible for the *OLR1* effects, the levels of *OLR1* expression were assessed in individuals bearing different genotypes. Expression of *OLR1* was reduced in AA individuals compared with CC and AC individuals, suggesting that the A at position 8,232 may be the nucleotide causing decreased *OLR1* expression, or it may be in linkage disequilibrium with a nearby functional SNP. Heart tissues were chosen for this study because of the role of *OLR1* in lipid metabolism in cardiac muscle tissues. Initial identification of *OLR1* was in bovine aortic endothelial cells where it is highly expressed compared with other tissues (Sawamura et al., 1997). There are 2 origins of milk lipids; one is fatty acid synthesis de novo in the mammary gland and the other is uptake of blood lipids by the mammary gland (Dils, 1986). Fatty acids imported to the mammary gland reflect the composition of fatty acids synthesized by other organs and released into the blood (Dils, 1986). Thus, the association of allele C with a significant increase in milk fat yield and milk fat percentage might reflect the high expression of *OLR1* synthesized in hearts of CC individuals and released into the blood.

It is noteworthy that 2 independent studies on the human gene reported that a SNP (C/T) in the 3'-UTR at position 1,073 (GenBank accession no. NM_002543) was associated with Alzheimer's disease. Luedecking-Zimmer et al. (2002) reported that, among 3 SNP identified in *OLR1*, the 3'-UTR polymorphism showed the most significant association with Alzheimer's disease. Moreover, they showed that the C allele at this position had a higher affinity for binding regulatory proteins compared with the T allele. Lambert et al. (2003) presented additional evidence that the 3'-UTR polymorphism was associated with Alzheimer's disease. Using electrophoretic mobility shift assays, they found that

the C allele was associated with higher binding affinity of nuclear proteins. In addition, they showed that the expression level of *OLR1* was lower in individuals bearing CC genotypes compared with CT and TT individuals. The location of SNP 1,073 in the human gene is 190 nucleotides downstream from the stop codon and the location of SNP 8,232 in the bovine gene is 223 nucleotides downstream from the stop codon.

The exact mechanism by which the bovine *OLR1* variants affect milk fat yield and milk fat percentage is not clear. However, given that *OLR1* is a receptor for oxLDL and that it is expressed abundantly in heart tissues, it might directly affect the metabolism of oxLDL, which in turn affects fat metabolism. In fact, the hypothesis that human *OLR1* variants might be involved in heart disease was tested in two independent studies. Mango et al. (2003) showed that the 3'-UTR SNP was associated with higher risk of developing acute myocardial infarction. Chen et al. (2003) reported association of the 3'-UTR SNP with coronary artery disease. Moreover, using an electrophoretic mobility shift assay, they found that the 3'-UTR SNP affects the binding of a putative transcription factor in an allele-specific manner. Thus, the 3'-UTR SNP that affected milk fat yield and milk fat percentage in the current study might affect mRNA stability or translation of the *OLR1* transcript as was predicted for the human gene (Chen et al., 2003). There is growing evidence that 3'-UTR sequences are involved in the regulation of gene expression and they can control stability of mRNA, polyadenylation, rates of translation, nuclear transport, and gene silencing (Conne et al., 2000). Recently, Oliver et al. (2005) reported that a 3'-UTR polymorphism in the *Gpc3* gene, a candidate gene chosen based on QTL studies, affects growth in mice.

Estimation of allele frequencies of *OLR1* in different cattle breeds provided additional support for the hypothesis that SNP 8,232 is linked to fat yield and percentage. It has long been known that *Bison bison*, and the Brown Swiss, Jersey, and Guernsey breeds have higher milk fat percentage than does the Holstein breed. Surprisingly, in this study it was found that the frequency of the C allele of SNP 8,232—the allele associated with an increase in fat yield and fat percentage in this Holstein population—was 54% in the Holsteins, whereas its frequency in *Bison bison*, Brown Swiss, and Jersey populations was 100%, and its frequency in Guernsey was 87%.

CONCLUSIONS

In conclusion, this study presents several lines of evidence that *OLR1* SNP 8,232 is linked to milk fat yield and percentage: i) significant effect of SNP 8,232;

ii) significant effect of haplotype 2, which differs from haplotype 1 only at position 8,232; iii) differences in expression of AA, AC, and CC genotypes at 8,232, primarily due to haplotypes 1 and 2, which differ only at 8,232; iv) high frequency of allele C in breeds with high fat percentage; v) lack of effect of SNP 7,160 and 7,161; and vi) effects of SNP 1073 in the 3'-UTR of the human gene.

We cannot completely exclude the possibility that the observed effects could be caused by linkage disequilibrium with other functional SNP in control regions of *OLR1* or by linkage disequilibrium with other nearby genes.

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