Diacylglycerol acyltransferase 1, stearoyl-CoA desaturase 1, and sterol regulatory element binding protein 1 gene polymorphisms and milk fatty acid composition in Italian Brown cattle

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ABSTRACT

Several lipogenic genes have been shown to have effects on lipid metabolism: stearoyl CoA desaturase 1 (*SCD1*) catalyzes the desaturation of several fatty acids (FA) in the *cis*- Δ^9 position in mammary glands of ruminant animals, diacylglycerol acyltransferase 1 (*DGAT1*) is a key enzyme in triacylglycerol synthesis in the mammary gland, and sterol regulatory element binding protein (*SREBP-1*) is a transcription factor that regulates expression levels of the *SCD1* gene and other genes relevant to lipid and FA metabolism in adipose tissue and mammary gland. In this work, 351 Italian Brown cows were genotyped for polymorphisms in the *SCD1*, *SREBP-1*, and *DGAT1* genes to reveal the allelic distribution in the population. Subsequently, effects on individual milk FA composition and on *cis*-9 unsaturated/saturated FA ratios, a proxy of mammary stearoyl CoA desaturase activity, were investigated. The genotypes of *SCD1* (A293V) and *DGAT1* (K232A) were determined by an approach based on the ligation detection reaction and a universal array, whereas the genotype of *SREBP-1* (84-bp insertion-deletion) was revealed by PCR amplification of intron 5. The genotype analysis showed an unbalanced distribution of alleles within all genes, being the allele with higher gene frequency at 82, 84, and 98% for *SCD1*, *SREBP-1*, and *DGAT1*, respectively. Significant associations between *SCD1* and *DGAT1* polymorphisms and milk FA composition were found, whereas *SREBP-1* polymorphism was not associated with milk FA composition. In particular, *SCD1* showed significant association with C14:1 *cis*-9 and C14:1 *cis*-9/C14:0, which is considered the best proxy of the desaturation activity in mammary gland. The *DGAT1* polymorphism had the strongest association with milk FA composition, which confirmed the key role of *DGAT1* in lipid metabolism of mam-

mary gland. However, the unbalanced distribution of alleles in all polymorphisms investigated suggested that the size of population should be increased to confirm the results of the present study.

Key words: stearoyl CoA desaturase (*SCD1*), sterol regulatory element binding protein 1 (*SREBP-1*), diacylglycerol acyltransferase 1 (*DGAT1*), milk fatty acid

INTRODUCTION

Milk fat is characterized by high amounts of saturated fatty acids (**SFA**) and low amounts of polyunsaturated fatty acids (**PUFA**). Epidemiologic, clinical, and animal studies indicate that high quantities of dietary SFA, as in ruminant milk, are associated with an increase in blood cholesterol and, consequently, with an increased risk of atherosclerosis and coronary heart disease in humans (Kromhout et al., 2002). These effects are due exclusively to lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0), whereas other SFA (C4:0 to C10:0 and C18:0) are neutral (Bonanome and Grundy, 1988). On the other hand, ruminant milk contains several compounds of great interest for human health, such as monounsaturated fatty acids (**MUFA**; mainly oleic acid, C18:1 *cis*-9) and conjugated linoleic acids (**CLA**). With regard to human health aspects, increasing the amount of unsaturated fatty acids in milk may be an important selection objective, but traditional selection programs based on performance recording and prediction of breeding values could be limited by the high cost of measuring the phenotypes. Recently, however, Soyeurt et al. (2006) showed that prediction of fatty acid (**FA**) concentrations in cow's milk was possible by mid-infrared spectrometry, a tool used during routine milk recording to predict different components of milk. Recent studies have suggested that genetic improvement of the milk nutritional quality based on the FA profile may be possible (Schennink et al., 2007; Soyeurt et al., 2007; Mele et al., 2009). The use of functional mutations that explain a significant proportion of the phenotypic variance is the most ef-

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ficient option for marker-assisted selection (Dekkers, 2004). Milk FA synthesis is regulated by several key enzymes (e.g., acetyl CoA carboxylase, fatty acid synthase, acyltransferases, stearoyl CoA desaturase 1). In some cases, several polymorphisms associated with specific effects on FA composition have been reported (Bionaz and Loor, 2008; Ibeagha-Awemu et al., 2008). Recently, polymorphisms in 2 genes coding for key enzymes of mammary lipid metabolism have been associated with a significant proportion of variation in milk FA composition in different cattle populations: stearoyl CoA desaturase 1 (*SCD1*) and diacylglycerol acyltransferase 1 (*DGAT1*; Mele et al., 2007; Schennink et al., 2008). Moreover, alternative genotypes of sterol regulatory element binding protein 1 (*SREBP-1*) have been associated with FA composition of intramuscular fat in Japanese Black cattle (Hoashi et al., 2007).

The *SCD1* gene is located on BTA 26 (Schennink et al., 2008). The *SCD1* enzyme catalyzes the conversion of C10:0–C18:0 SFA into their MUFA counterparts and is also involved in the synthesis of CLA in the mammary glands of ruminants (Corl et al., 2001). Three SNP in complete linkage disequilibrium, which result in 2 haplotypes, have been detected in exon 5 (Taniguchi et al., 2004). The third SNP causes the substitution of valine (allele V) to alanine (allele A) on residue 293 (A293V). Several studies reported significant association between A293V genotypes and milk FA composition in intramuscular fat (Taniguchi et al., 2004) and mammary gland (Mele et al., 2007; Schennink et al., 2008; Kgwatalala et al., 2009).

The *DGAT1* gene is located on BTA14 (Grisart et al., 2002). The *DGAT1* enzyme plays a key role in triacylglycerol synthesis: it catalyzes the esterification of a fatty acyl-CoA to the *sn*-3 position of a diacylglycerol. A lysine to alanine polymorphism in *DGAT1* (K232A), explaining 50% of the genetic variation in milk fat percentage, has been reported to be strongly associated with milk FA composition in Holstein cattle (Schennink et al., 2007). The *DGAT1* K allele has been associated with a higher content of C16:0 and lower contents of C14:0, unsaturated C18, and CLA (Schennink et al., 2007). Moreover, Grisart et al. (2002) have confirmed that the K232A polymorphism in *DGAT1* is responsible for a QTL underlying several milk production traits.

The *SREBP-1* gene is located on BTA19 (Hoashi et al., 2007) and encodes the transcription factor that regulates gene expression levels of *SCD1* and other genes of lipid metabolism and it is involved in the regulation of bovine mammary lipid synthesis (Harvatin and Bauman, 2006). An 84-bp insertion/deletion (*indel*) polymorphism that caused the presence of a long (L-type, 84-bp insertion) and short (S-type, 84-bp deletion) allele (Hoashi et al., 2007), was revealed in

intron 5. Hoashi et al. (2007) revealed a 1.3% higher content of MUFA in intramuscular fat of Japanese Black cattle with SS genotypes compared with those having LL genotypes.

The objective of this study was to genotype the *SCD1*, *DGAT1*, and *SREBP-1* genes in Italian Brown cattle and to investigate the association between the genotypes and milk FA composition.

MATERIALS AND METHODS

Milk and Blood Samples

Individual morning milk samples from 351 Italian Brown cows were collected. Sampling was conducted in 16 dairy herds (22 ± 6 cows for each herd) that were similar in feeding (no pasture, TMR with maize silage and alfalfa hay as forage basis, forage:concentrate ratio ranging from 50:50 to 60:40) and farming system and located in northeastern Italy. The cows belonged to 21 half-sib families (20 ± 8 daughters per bull). Samples were collected from June 2006 to May 2007. We collected samples with a SCC <300,000 cells/mL to exclude any effects of mastitis. A single milk sample from each cow was analyzed to define FA composition (described later). Blood samples were collected in EDTA vacutainers (BD Vacutainer Systems, Plymouth, UK). Milk and blood samples were stored at -20°C until analysis. Data regarding parity number, calving date, morning milk yield, and days in milk were recorded for each cow.

Phenotypes

The variables considered were individual FA from C8:0 to C22:5 n-3 and the following classes of FA: SFA, MUFA, PUFA, medium-chain FA (FA with carbon chain length from 8 to 17 C), long-chain FA (FA with carbon chain length >17 C); *trans* FA (all *trans* isomers); branched-chain FA (C14:0 *iso*, C15:0 *iso*, C15:0 *anteiso*, C16:0 *iso*, C17:0 *iso*, C17:0 *anteiso*), and odd-chain FA (**OCFA**: C11:0, C13:0, C15:0, C17:0).

The extent of FA desaturation was determined by calculating the ratio of *cis*-9 unsaturated to *cis*-9 unsaturated + saturated for the different FA that *SCD1* catalyzes (Palmquist et al., 2004). The following ratios were calculated:

- C14:1 *cis*-9 to C14:1 *cis*-9 + C14:0: desaturation index for C14:0 (**DI 14**);
- C16:1 *cis*-9 to C16:1 *cis*-9 + C16:0: desaturation index for C16:0 (**DI 16**);
- C18:1 *cis*-9 to C18:1 *cis*-9 + C18:0: desaturation index for C18:0 (**DI 18**);

- C18:2 *cis*-9, *trans*-11 to C18:2 *cis*-9, *trans*-11 + C18:1 *trans*-11: desaturation index for C18:1 *trans*-11 (**RA/VA**).

These indices are a proxy of the desaturase activity (Bernard et al., 2006). Moreover, a general index of desaturation (**DI**) was calculated with the following formula (adapted from Malau-Aduli et al., 1997):

$$\text{DI} = (\text{C14:1 } cis\text{-9} + \text{C16:1 } cis\text{-9} + \text{C18:1 } cis\text{-9} + \text{C18:2 } cis\text{-9, } trans\text{-11}) / (\text{C14:1 } cis\text{-9} + \text{C16:1 } cis\text{-9} + \text{C18:1 } cis\text{-9} + \text{C18:2 } cis\text{-9, } trans\text{-11} + \text{C14:0} + \text{C16:0} + \text{C18:0} + \text{C18:1 } trans\text{-11}).$$

Genetic Analysis

The GFX Genomic Blood DNA Purification kit (Amersham Biosciences, Piscataway, NJ) was used for DNA extraction directly from blood. Extraction was performed starting with 300 μL of blood to recover at least 5 ng/ μL of genomic DNA at a final volume of 100 μL . The primers used for the amplification of the 3 genes were designed using Primer3 software (Rozen and Skaletsky, 2000) and are listed in Table 1.

Genotypes for the *DGAT1* and the *SCD1* were analyzed using the ligation detection reaction–universal array (**LDR-UA**) approach described in Chessa et al. (2007). The technique usually requires 3 probes for each SNP to be genotyped: 2 discriminating probes each carrying a different nucleotide at its 3' end (corresponding to the 2 bases of the biallelic SNP) and a different fluorescent dye at its 5' end, and a common probe whose sequence starts with the nucleotide located immediately after the SNP. Primers and probes used to genotype the *DGAT1* and *SCD1* genes are reported in Table 1 and were previously tested on Brown Swiss cattle (Chessa et al., 2008). The DNA with different microarray spots was sequenced by PRIMM srl (Milan, Italy), to verify the occurrence of the SNPs. Two samples per putative genotype were randomly chosen. Primers used for sequencing were the same reported in Table 1.

To distinguish S-types (84-bp deletion) and L-types (84-bp insertion) of *SREBP-1*, a PCR for the amplification of a fragment of intron 5 was performed in a 15- μL reaction mixture containing 2 μL of DNA solution (100 to 200 ng), 1 $\times$ PCR Master Mix (Fermentas, Vilnius, Lithuania), and 6 pmol of each primer. The following conditions were used for the PCR assay: an initial denaturation step of 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, 56°C for 45 s, 72°C for 45 s, and a final extension step of 72°C for 7 min using a PTC-200 DNA Engine Thermal Cycler (MJ Research Inc., Waltham,

Table 1. Primer and ligase detection reaction–universal array (LDR-UA) probe sequences, predicted sizes of PCR products, and reference sequences for the amplification of stearoyl-CoA desaturase 1 (*SCD1*), diacylglycerol acyltransferase 1 (*DGAT1*), and sterol regulatory element binding protein 1 (*SREBP-1*) gene polymorphism target fragments and the identification of the *SCD1* and *DGAT1* polymorphisms with the LDR-UA technology

Name	Sequence ¹ (5' → 3')	DNA target	Amplicon size, bp	Reference sequence GenBank accession no.
<i>SCD</i> Fw	CAGTCCTTGCTCCACCACTT	<i>SCD</i> exon 5	725	AY241932
<i>SCD</i> Rv	AGCATTTGTGGCTTGCTCTT	<i>SCD</i> exon 5		
<i>DGAT1</i> Fw	CCATCTCTCTCTCAAGCTG	<i>DGAT1</i> exon 8	381	AJ318490
<i>DGAT1</i> Rv	GGGAAGTTGAGCTCGTAGCA	<i>DGAT1</i> exon 8		
<i>SREBP-1</i> Fw	ATCGAGAAACGCTACCGC	<i>DGAT1</i> exon 8	408/492	NW_001493671
<i>SREBP-1</i> Rv	TCACTCCACTTTATGCTCTAC	<i>SREBP-1</i> intron 5		(Region 672111–689592)
<i>SCD-10329-C</i>	Cy3-CCCCGAGAGAAATATCTGTGTTCCCTGGGAGCTGC	<i>SREBP-1</i> intron 5		AY241932
<i>SCD-10329-T</i>	Cy5-CCCCGAGAGAAATATCTGTGTTCCCTGGGAGCTGT	SNP 10329		
<i>SCD-Common Probe</i>	GGTAAGTCAGCAGTCCACAGCAAGACCAACGCTCT			
<i>DGAT1-10433/4-GC</i>	Cy3-CCGCTTGCTGCTAGCTTTGGCAGGTAAGGC			
<i>DGAT1-10433/4-AA</i>	Cy5-GCGCTTGCTGCTAGCTTTGGCAGGTAAGAA			
<i>DGAT1-Common Probe</i>	GGCCAAACGGGGAGCTGCCAGC	SNP 10433/4		AJ318490

¹Fluorescent dye labeling the discriminating probes at their 5' ends. Cy3 = cyanine 3; Cy5 = cyanine 5.

Figure 1. Genotyping of the sterol regulatory element binding protein 1 (*SREBP-1*) 84-bp insertion/deletion (indel) polymorphism. L = long type (84-bp insertion); S = short type (84-bp deletion); LL and SS genotypes are characterized by the presence of 492-bp and 408-bp PCR fragments, respectively, whereas the presence of both fragments indicates the LS genotype.

MA). The PCR products were run on a 2% agarose gel as shown in Figure 1.

The occurrence of the 84-bp indel (characterizing the short and long types of *SREBP-1*) in the analyzed samples was confirmed by sequencing 2 homozygous samples with the putative 84-bp insertion and 2 homozygous samples with the putative 84-bp deletion. The samples were sequenced by PRIMM Srl using the primers described above (Table 1).

Nucleotide sequences of all genes were analyzed by using Bioedit software (Hall, 1999).

Milk FA Analysis

Milk fat was extracted as reported in Mele et al. (2007). Methyl esters of FA were prepared by Christie's alkali catalyzed transmethylation procedure (Christie, 1982) with nonadecanoic acid methyl ester (Sigma Chemical Co., St. Louis, MO) as the internal standard. The FA composition was determined by GC using a ThermoQuest gas chromatograph (Milan, Italy) equipped with a flame-ionization detector and a high polar fused-silica capillary column (Chrompack CP-Sil 88 Varian, Middelburg, the Netherlands; 100 m, 0.25 mm i.d.; film thickness 0.20 μ m). Helium was used as the carrier gas at a flow of 1 mL/min. The split ratio was 1:100. An aliquot of the sample was injected under the following GC conditions: the oven temperature was brought to 150°C and held at that level for 1 min; it was then increased to 185°C at a rate of 5°C/min, and held at that level for 20 min, before being once again increased to 188°C at 0.3°C/min and held for 1 min, and then to 230°C at a rate of 3°C/min, and held for 15 min. The injector temperature was set at 270°C and the detector temperature was set at 300°C. *Cis* and *trans* C18:1 isomers were determined on a second aliquot of the same sample at 175°C (isothermal step) for 65 min using the same capillary column. Individual FA methyl esters were identified by comparison to a standard mixture of 52 Component FAME Mix (Nu-Chek Prep Inc., Elysian, MN). The identification of isomers of C18:1

was based on commercial standard mixtures (Supelco, Bellefonte PA) and published isomeric profiles (Kramer et al., 2004). Milk FA composition was expressed as grams per 100 grams of fat.

Statistical Analysis

Data were analyzed with a mixed linear model including herd, parity, calving season, DIM, and genotype as fixed effects and sire as random effect (SAS Institute, 1999):

$$y_{ijklmn} = \mu + \text{HERD}_i + \text{PARITY}_j + \text{DIM}_k + \text{SEASON}_l + \text{GENOTYPE}_m + \text{SIRE}_n + e_{ijklmn}, \quad [1]$$

where y_{ijklmn} = dependent variable (milk FA as g/100 g of lipids, FA ratios); μ = overall mean; HERD_i = fixed effect of the i th herd (1...16); PARITY_j = fixed effect of the j th parity (first, second, $\geq$ third); DIM_k = fixed effect of the k th days in milking interval (<100, 100 to 200, >200); SEASON_l = fixed effect of the l th calving season (winter, spring, summer, autumn); GENOTYPE_m = fixed effect of the m th genotype (i.e., AA, AV, VV for *SCD1* gene; LL, LS, and SS for *SREBP-1* gene; AA and AK for *DGAT1* gene); SIRE_n = random effect of the n th sire (21); and e_{ijklmn} = random residual.

All interactions among fixed variables were included in the initial model and were eventually dropped from the final model when they were not significant. Results on the 3 genotype effects were presented as least squares means $\pm$ standard errors, and linear contrasts were tested between homozygote and heterozygote genotypes by t -test with Tukey's adjustment within each SNP. Differences between means were declared significant at $P \leq 0.05$. In the case of the *DGAT1* genotype, linear contrasts between AA and AK genotypes were tested, because only 2 animals with the KK genotype were found. The average gene substitution effect for the *SCD1* and *SREBP-1* loci on milk FA content was calculated according to Falconer and McKay (1996):

$$\alpha = a + d(q - p), \quad [2]$$

where α is the gene substitution effect, a is the phenotypic value of the homozygote genotype, d is the phenotypic value of the heterozygote genotype, and p and q are the allelic frequencies.

The contributions of the *SCD1*, *SREBP-1*, and *DGAT1* loci to the variability of the traits considered were assessed by calculating the reduction of residual variance observed in the full model [1], compared with a reduced model that did not include the *SCD1*,

Table 2. Distribution of stearoyl-CoA desaturase (*SCD1*), sterol regulatory element binding protein 1 (*SREBP-1*), and diacylglycerol acyltransferase 1 (*DGAT1*) genotypes in the 351 Italian Brown cattle

Gene	Polymorphism	Genotype	Animals per genotype, n	Relative frequency, %
<i>SCD1</i>	A293V	AA	15	4
		AV	102	29
		VV	234	67
<i>SREBP-1</i> 84-bp indel ¹	84-bp indel	LL	252	72
		LS	86	24
		SS	13	4
<i>DGAT1</i>	K232A	AA	338	96
		AK	11	3
		KK	2	1

¹L = long type (84-bp insertion); S = short type (84-bp deletion).

SREBP-1, and *DGAT1* genotypes as fixed factors (Neter et al., 1996). Hardy-Weinberg equilibrium was evaluated by chi-square test.

Using data from this study for Italian Brown Swiss and data from a previous work (Mele et al., 2007) for Italian Holstein, population inbreeding coefficient (F_{IS}) was calculated, to evaluate the different selection pressure on these breeds related to *SCD1* polymorphism, according to the following formula (Wright, 1951):

$$F_{IS} = (H_E - H_O)/H_E, \quad [3]$$

where F_{IS} = inbreeding coefficient; H_E = expected heterozygosity ($= 2pq$; where p and q are allelic frequencies of V and A *SCD1* types, respectively); H_O = observed heterozygosity. The statistic F_{IS} is the classical Wright's *F*-statistic, which estimates the variation within populations. In particular it measures the reduction in heterozygosity in an individual caused by non-random mating within its subpopulation. The genetic variability between Italian Brown (data of this work), Italian Holstein (data from Mele et al., 2007), Jersey (data from Kgwatalala et al., 2009), and Piedmontese (data from Moioli et al., 2007) was calculated by F_{ST} with the following formula (Wright, 1951):

$$F_{ST} = (H_T - H_S)/H_T, \quad [4]$$

where F_{ST} = inbreeding coefficient among subpopulation within the total population; H_T = expected heterozygosity of an individual in a randomly mating total population (given the average allele frequency of the total population $= 2p_iq_i$); and H_S = expected heterozygosity of an individual in a randomly mating subpopulation with the same allele frequencies (averaged over subpopulations $= 2 p_i q_i$). F_{ST} is another Wright's *F*-statistic, which estimates the variation caused by differences among populations; it represents the reduction in heterozygosity of a subpopulation due to genetic drift (Wright, 1951).

RESULTS AND DISCUSSION

Genotype of Lipogenic Genes

Polymorphisms of lipogenic genes in dairy cattle have been studied in several breeds (Kaupe et al., 2004; Taniguchi et al., 2004; Moioli et al., 2007; Morris et al., 2007; Milanesi et al., 2008; Kgwatalala et al., 2009). In this study the genotype frequencies of 3 key genes of lipid metabolism (*SCD1*, *DGAT1*, and *SREBP-1*) in Italian Brown cattle were reported.

The genotype analysis identified the 3 nonsynonymous SNP in exon 5 of *SCD1* that cause the substitution of valine with alanine in residue 293 (A293V), as described by Taniguchi et al. (2004). Sequence analysis revealed that the 3 SNP were in linkage disequilibrium and constituted the 2 haplotypes (A and V types) described in literature (Taniguchi et al., 2004; Mele et al., 2007; Milanesi et al., 2008). On the basis of the observed *SCD1* genotype frequencies in this sample of Italian Brown cows (Table 2), the V allele was present at a higher frequency than the A allele (82% vs. 18% for V and A, respectively). The results did not agree with Milanesi et al. (2008), who revealed a low gene frequency of the V allele in Italian Brown (15%). Previous studies reported a higher frequency of the A allele in Italian Holstein, Jersey, Valdostana, and Japanese Black cattle breeds (Taniguchi et al., 2004; Mele et al., 2007; Moioli et al., 2007; Kgwatalala et al., 2009). Only in the Piedmontese breed (Moioli et al., 2007) was the frequency of the V allele found to be higher than that of the A allele (58% and 42% for V and A allele, respectively). In this study, *SCD1* genotypes were in Hardy-Weinberg equilibrium (chi-square $P = 0.60$). At this time, we cannot assess if the higher V allele frequency in the Italian Brown population is because of an effect of genetic selection or is due to other causes. However, data reported in Table 3 revealed different F_{IS} coefficients between Italian Holstein (data from Mele et al., 2007) and Italian Brown. In particular, Italian Brown had a higher F_{IS} than Italian Holstein. Moreover, the F_{IS} value was posi-

Table 3. Expected (H_E) and observed (H_O) heterozygosity and inbreeding coefficient (F_{IS}) of A293V polymorphism of the stearoyl-CoA desaturase (*SCD1*) gene in Italian Holstein (Mele et al., 2007) and Italian Brown¹

Breed	n	f(A)	f(V)	H_E	H_O	F_{IS}
Italian Holstein	297	0.57	0.43	0.49	0.60	-0.23
Italian Brown	351	0.18	0.82	0.30	0.29	0.05
Total	648	0.37	0.64	0.40	0.43	-0.09

¹f(A) and f(V) represent the A and V type frequencies, respectively.

tive for Italian Brown and negative for Italian Holstein, suggesting a heterozygote deficiency in the first breed. Recently, positive associations among the V allele and milk and protein yields have been reported in Holstein cattle (Macciotta et al., 2008). To evaluate whether the effects of selection or genetic drift have contributed to the shaping of allelic frequencies for the *SCD1* locus, we calculated the F_{ST} coefficient to estimate the distance between Italian Brown and 2 dairy breeds (Italian Holstein, allelic frequencies obtained from Mele et al., 2007; Jersey, allelic frequencies obtained from Kgwatalala et al., 2009) as well as Italian Brown and an Italian beef breed (Piedmontese, allelic frequencies obtained from Moiola et al., 2007). Data reported in Table 4 revealed moderate or high F_{ST} values between Italian Brown and Italian Holstein (0.16) and between Italian Brown and Jersey (0.40), whereas a low F_{ST} value was obtained between Italian Holstein and Jersey (0.07). High F_{ST} values indicate an elevated distance between Italian Brown and the other dairy breeds considered, but cannot explain if the distance was caused by genetic drift or a different selection.

Sequence analysis revealed the presence of a new SNP in intron 5 (10436° residue A→G or A10436G), which was not reported in literature (position 10436 corresponds to the position 308 in the sequence reported in Figure 2). Our results also suggested that this new polymorphism should be in complete linkage disequilibrium with the above 2 haplotypes (G was related to A type, and A was related to V type).

For the *SREBP-1* gene, we identified an 84-bp indel polymorphism in intron 5 (Figure 1), as reported by Hoashi et al. (2007). Allele frequencies have shown a higher distribution of the L-type (84-bp insertion) than the S-type (84-bp deletion) as reported in Table 2 (84% vs. 16%). A higher L-type frequency was also reported by Hoashi et al. (2007) for Japanese Black cattle (63%) and for Holsteins (100%). Genotypes were in Hardy-Weinberg equilibrium (chi-square $P = 0.28$). Although according to the literature only a few cattle breeds have been genotyped, the greatest spread of the L-type could suggest that it is the ancestral allele. The alignment of the sequenced sample for *SREBP-1* with the refer-

ence sequence (GenBank accession no. NW_001493671, region 672111–689592) and that published by Hoashi et al. (2007) for the long and short forms of intron 5 (GenBank accession nos. AB355704 and AB355705) showed that the short form is also characterized by a C → G transversion (Figure 3) 7 nucleotides before the deletion and that the long form of the Japanese Black cattle is characterized by a C → T transition at the 17th nucleotide of the insertion, showing greater variability than that described to date.

In the present study of Italian Brown cattle, the K232A polymorphism as described by Grisart et al., (2002) in Holstein cattle was also present. Genotypic frequencies are reported in Table 2. The frequency of A type was 98% and the population was in Hardy-Weinberg equilibrium (chi-square $P = 0.43$). The KK genotype was found in only 2 cows. The frequency of the A allele was reported in a wide range of *Bos taurus* breeds, and the values ranges between 0.22 and 1.00 (Spelman et al., 2002; Kaupe et al., 2004; Sanders et al., 2006; Gautier et al., 2007; Näslund et al., 2008). Only in Holstein cattle was a more balanced distribution of the 2 alleles revealed: 60% versus 40% for A and K, respectively (Banos et al., 2008; Schennink et al., 2008).

It is very interesting to reveal the different allele distributions between Italian Brown and Holstein cattle for the 3 genes considered. These mutations may have occurred early in the history of cattle domestication, and the great variation observed for the frequencies of wild-type alleles (V for *SCD1*, L for *SREBP-1*, K for *DGAT1*) reflects different breeding objectives regarding milk composition in different countries and breeds (Gautier et al., 2007). For example, the frequency of the ancestral K allele at the K232A polymorphism has increased only recently, in particular in the Holstein breed, possibly because of more pronounced selection favoring fat content (Gautier et al., 2007).

Table 4. Expected heterozygosity of an individual in a randomly mating total population (H_T), expected heterozygosity of an individual in a mating breed (H_S), and inbreeding coefficient among subpopulation within the total population (F_{ST}) of A293V polymorphism of the stearoyl-CoA desaturase (*SCD1*) gene in several breeds¹

Breed	H_S	H_T	F_{ST}
Italian Brown-Italian Holstein	0.39	0.47	0.16
Italian Brown-Jersey	0.30	0.50	0.40
Italian Brown-Piedmontese	0.39	0.42	0.07
Italian Holstein-Jersey	0.40	0.43	0.07
Italian Holstein-Piedmontese	0.49	0.50	0.02
Piedmontese-Jersey	0.40	0.47	0.16

¹Italian Holstein (data from Mele et al., 2007); Italian Brown (data of this work); Jersey (data from Kgwatalala et al., 2009); and Piedmontese (data from Moiola et al., 2007).

AY241932	1	CTTCATCCTG	CCCACACTCG	TGCCATGGTA	TCTGTGGGAT	GAAACGTTTC	AAAAACAGCCT
Genotype AA1	1	*****	*****	***G***	*****	*****	*****
Genotype AA2	1	*****	*****	***G***	*****	*****	*****
Genotype AV1	1	*****	*****	***N***	*****	*****	*****
Genotype AV2	1	*****	*****	***N***	*****	*****	*****
Genotype VV1	1	*****	*****	***A***	*****	*****	*****
Genotype VV2	1	*****	*****	***A***	*****	*****	*****
AY241932	61	GTTTTTTGCC	ACCTTATTCC	GTTATGCCCT	TGGGCTCAAC	GTCACCTGGC	TGGTGAATAG
Genotype AA1	61	*****	*****	***C***	*****	*****	*****
Genotype AA2	61	*****	*****	***C***	*****	*****	*****
Genotype AV1	61	*****	*****	***N***	*****	*****	*****
Genotype AV2	61	*****	*****	***N***	*****	*****	*****
Genotype VV1	61	*****	*****	***T***	*****	*****	*****
Genotype VV2	61	*****	*****	***T***	*****	*****	*****
AY241932	121	TGCTGCCCCAT	ATGTATGGAT	ACCGCCCTTA	TGACAAGACC	ATCAACCCCC	GAGAGAATAT
Genotype AA1	121	*****	*****	*****	*****	*****	*****
Genotype AA2	121	*****	*****	*****	*****	*****	*****
Genotype AV1	121	*****	*****	*****	*****	*****	*****
Genotype AV2	121	*****	*****	*****	*****	*****	*****
Genotype VV1	121	*****	*****	*****	*****	*****	*****
Genotype VV2	121	*****	*****	*****	*****	*****	*****
AY241932	181	TCTGGTTTCC	CTGGGAGCTG	CGGGTAAGTC	AGCAGTCCAC	AGCAAGACCA	CGTCTAGTGG
Genotype AA1	181	*****	*****	T*****	*****	*****	*****
Genotype AA2	181	*****	*****	T*****	*****	*****	*****
Genotype AV1	181	*****	*****	N*****	*****	*****	*****
Genotype AV2	181	*****	*****	N*****	*****	*****	*****
Genotype VV1	181	*****	*****	C*****	*****	*****	*****
Genotype VV2	181	*****	*****	C*****	*****	*****	*****
AY241932	241	TCTGCTGCTT	AGGGTATTAG	GTTACGTGCC	AGAAAAACTA	GATTTACCTG	TTTATGACC
Genotype AA1	241	*****	*****	*****	*****	*****	*****
Genotype AA2	241	*****	*****	*****	*****	*****	*****
Genotype AV1	241	*****	*****	*****	*****	*****	*****
Genotype AV2	241	*****	*****	*****	*****	*****	*****
Genotype VV1	241	*****	*****	*****	*****	*****	*****
Genotype VV2	241	*****	*****	*****	*****	*****	*****
AY241932	301	CCTCTCCATA	TGTCATTCCA	CTATAAAACT	AAGGGACAGT	ATTAGAAAAC	CCTTGAAAAGT
Genotype AA1	301	*****G**	*****	*****	*****	*****	*****
Genotype AA2	301	*****G**	*****	*****	*****	*****	*****
Genotype AV1	301	*****N**	*****	*****	*****	*****	*****
Genotype AV2	301	*****N**	*****	*****	*****	*****	*****
Genotype VV1	301	*****A**	*****	*****	*****	*****	*****
Genotype VV2	301	*****A**	*****	*****	*****	*****	*****
AY241932	361	TAAGCAACAA	GTCCTATGTA				
Genotype AA1	361	*****	*****				
Genotype AA2	361	*****	*****				
Genotype AV1	361	*****	*****				
Genotype AV2	361	*****	*****				
Genotype VV1	361	*****	*****				
Genotype VV2	361	*****	*****				

Figure 2. Sequence alignment of the 2 AA (genotype AA1 and AA2), AV (genotype AV1 and AV2), and VV genotype (genotype VV1, VV2) sequenced samples. Identical nucleotides are replaced by an asterisk (*) and the substitutions are indicated by letter. The simultaneous presence of both nucleotides (heterozygous genotype) is indicated with a N.

Effects of *SCD1*, *DGAT1*, and *SREBP-1* Genotypes on Milk FA Composition

The *SCD1* genotype was significantly associated with C14:1 *cis*-9 and DI 14 ($P \leq 0.01$) (Table 5). In par-

ticular, the VV genotype was associated with 18.3% and 20.6% more C14:1 *cis*-9 and DI 14, respectively, compared with the AA genotype (Table 5). The average gene substitution effect for *SCD1* was 0.05% and 0.004% for C14:1 *cis*-9 and DI 14 respectively. In


```

NW_001493671 1 GTGTGGGCTG AGGCCCTAAC AGGGTGGCTC TGGGCAAGCA GGCACCTGGG AGGAGGAGGA
AB355704      1 *****
LF1          1 *****
LF2          1 *****
AB355705     1 *****
SF1          1 *****
SF2          1 *****

NW_001493671 61 GGAAGATGGG GCTGGGCAGA CAGTCCTGGG GCCCCAGCCT CCTCGGGCCT GGCAGCTCTG
AB355704      61 *****T*****
LF1          61 *****
LF2          61 *****
AB355705     61 *****G*****
SF1          61 *****G*****
SF2          61 *****G*****

NW_001493671 121 TTCAGCTGAG CTTCAGGGAA GCCCGGGGTG GCACAGGCTC TTCCAGGTGC TGGGGATTTA
AB355704      121 *****
LF1          121 *****
LF2          121 *****
AB355705     121 -----
SF1          121 -----
SF2          121 -----

NW_001493671 181 GCAGGGGACA GACAGAGATC CTGCTGTTGA GTAGCTGACG TTCTAGCAGG GTGCGGGGGC
AB355704      181 *****
LF1          181 *****
LF2          181 *****
AB355705     181 *****
SF1          181 *****
SF2          181 *****

```

Figure 3. Sequence alignment of the 2 long-form, homozygous sequenced samples (LF1 and LF2 are the names of 2 samples of the genotyped cows), and of the 2 short-form, homozygous sequenced samples (SF1 and SF2 are the names of 2 samples of the genotyped cows) of the fifth intron of sterol regulatory element binding protein 1 (*SREBP-1*) with the Japanese Black cattle sequence published by Hoashi et al. (2007) (GenBank accession no. AB355704 and AB355705) and the reference sequence (GenBank accession no. NW_001493671; region 672111–689592). Identical nucleotides are replaced by an asterisk (*), deleted nucleotides are indicated with a dash (-), and the substitutions are indicated by letter. The alignment continued with no mutation until nucleotide 404 (not shown).

terms of explained variance, the contribution of *SCD1* genotype to C14:1 *cis*-9 variation in milk fat was quite low (4.35%; Table 6), in agreement with the results reported in Holstein cattle (Mele et al., 2007) and Japanese Black cattle (Taniguchi et al., 2004). In Holstein cattle, Mele et al. (2007), Schennink et al. (2008), and Kgwatalala et al. (2009) found a significant positive association among the A allele of *SCD1* and C14:1 *cis*-9 and DI 14. Moreover, in the literature, significant associations of the *SCD1* polymorphism were also reported with C10:1/C10:0, C12:1/C12:0 (Schennink et al., 2008; Kgwatalala et al., 2009), C18:1 *cis*-9/C18:0, RA/VA, and C16:1 *cis*-9/C16 ratios (Schennink et al., 2008). In agreement with Schennink et al. (2008) and Mele et al. (2007), *SCD1* polymorphisms in this study were not associated with DI. This finding suggests that *SCD1* polymorphisms should result in altered substrate specificity, supported by the differential effects on the individual FA ratios, instead of a variation of the enzyme activity. This is the first time that the V allele has been associated with a higher DI 14. Probably, *SCD1* is not the only genetic factor that controls desaturation expression or activity.

The relationship between *SCD1* polymorphism and milk C18:2 *cis*-9 *trans*-11 content is of particular interest. Milk C18:2 *cis*-9 *trans*-11 content is characterized by individual variation (up to 8-fold) that can be observed in cows fed the same diet (Bauman et al., 2001; Secchiari et al., 2003). Therefore, an important role of the genetic background of animals could be hypothesized. In this study, the AA genotype was associated with 7% more C18:2 *cis*-9 *trans*-11 compared with VV genotype; however, this difference did not reach a level of significance ($P = 0.19$), perhaps because of the small size of the sampled population. Mele et al. (2007) revealed the same association in Holstein cattle using a similar number of animals (297 vs. 351).

It has been shown that SREBP regulates gene transcription activation by binding to sterol regulatory element sequences contained in the promoter of its downstream genes, including *SCD1* (Hoashi et al., 2007). Therefore, differences in expression level or mutation(s) of the SREBP gene may affect the expression level of *SCD1*, leading to differences in FA composition in the fat tissue of cattle. According to Hoashi et al. (2007), the 84-bp indel polymorphism in intron 5 of the bovine

Table 5. Least squares means of selected measures of milk fatty acids (FA) content (g/100 g of total lipids) for different stearoyl-CoA desaturase (*SCD1*), sterol regulatory element binding protein 1 (*SREBP-1*), and diacylglycerol acyltransferase 1 (*DGAT1*) genotypes

Fatty acid	<i>SCD1</i>			Overall <i>P</i> -value
	AA	AV	VV	
	(n = 15)	(n = 102)	(n = 234)	
C14:1 <i>cis</i> -9	0.736 ± 0.065 ^A	0.779 ± 0.038 ^A	0.871 ± 0.032 ^B	0.001
DI 14 ¹	0.063 ± 0.004 ^A	0.066 ± 0.002 ^B	0.076 ± 0.001 ^C	<0.001
	<i>SREBP-1</i>			
	LL	LS	SS	
	(n = 252)	(n = 86)	(n = 13)	
C15:0	0.910 ± 0.024 ^A	0.973 ± 0.029 ^B	1.043 ± 0.064 ^B	0.019
Odd-chain FA	2.071 ± 0.043 ^a	2.152 ± 0.049 ^b	2.275 ± 0.101 ^c	0.048
	<i>DGAT1</i>			
	AA	AK		
	(n = 338)	(n = 11)		
C10:0	2.626 ± 0.054	2.252 ± 0.168		0.022
C14:1 <i>cis</i> -9	0.840 ± 0.036	1.049 ± 0.078		0.004
C16:0 <i>iso</i>	0.212 ± 0.004	0.171 ± 0.018		0.026
C16:1 <i>cis</i> -9	1.127 ± 0.027	1.466 ± 0.096		<0.001
C18:0	8.973 ± 0.125	7.293 ± 0.517		0.001
C18:1 <i>trans</i> -6-8	0.186 ± 0.004	0.215 ± 0.014		0.040
C18:1 <i>trans</i> -10	0.405 ± 0.018	0.642 ± 0.073		0.001
C20:0	0.097 ± 0.001	0.080 ± 0.006		0.008
C18:2 <i>cis</i> -9 <i>trans</i> -11	0.450 ± 0.009	0.536 ± 0.032		0.008
C24:0	0.025 ± 0.001	0.018 ± 0.003		0.026
DI 14	0.072 ± 0.002	0.087 ± 0.005		0.002
DI 16 ²	0.041 ± 0.001	0.050 ± 0.003		0.003
DI 18 ³	0.674 ± 0.004	0.707 ± 0.008		0.012
RA/VA ⁴	0.335 ± 0.004	0.383 ± 0.014		0.001

^{A-C}Means within rows with different superscripts differ ($P < 0.01$).

^{a-c}Means within rows with different superscripts differ ($P < 0.05$).

¹DI (desaturation index) 14 = C14:1 *cis*-9/C14:0 + C14:1 *cis*-9.

²DI 16 = C16:1 *cis*-9/C16:0 + C16:1 *cis*-9.

³DI 18 = C18:1 *cis*-9/C18:0 + C18:1 *cis*-9.

⁴RA/VA = rumenic acid/vaccenic acid; C18:2 *cis*-9 *trans*-11/C18:1 *trans*-11 + C18:2 *cis*-9 *trans*-11.

SREBP-1 gene could indirectly contribute to fat quality characteristics in cattle, affecting the level of translation efficiency of *SREBP-1* itself. Our findings showed that the *SREBP-1* polymorphism was not associated with milk FA composition (Table 5). Data reported in Table 5 show a higher DI 14 in cows with the SS genotype, but the difference did not reach significance ($P = 0.07$), probably because of the small number of animals ($n = 13$) with the SS genotype. In Japanese Black cattle, Hoashi et al. (2007) reported higher values of DI 14 and MUFA content in milk from SS cows. Moreover, we revealed higher content of C15:0 and OCFA in SS type than in LL type (gene substitution effects were 0.069 and 0.136 for C15:0 and OCFA respectively; Tables 5 and 6).

The association of *DGAT1* polymorphism with milk FA composition was revealed by comparing the AA and AK genotypes, because only 2 animals with the KK genotype were found. Statistical analysis showed a higher C14:1 *cis*-9 (+ 20%), C16:1 *cis*-9 (+ 23%), C18:1 *trans* 6-8 (+ 10%), C18:1 *trans*-10 (+ 38%), C18:2 *cis*-9, *trans*-11 (+ 17%) contents and lower C10:0 (−14%), C16:0 *iso* (−19%), C18:0 (−19%), C20:0 (−20%), and C24:0 (−34%) percentages for the AK type than for the homozygous AA type (Table 5). Moreover, AK cows showed higher values of all FA ratios with the exception of DI (Table 5). On the contrary, Schennink et al. (2008) revealed higher values of DI 18, RA/VA, and DI and lower values of DI 14, DI 16, C10:1/C10:0, and C12:1/C12:0 ratios for the AA and AK types. On the

Table 6. Mean (μ), phenotypic variance [V(p)], contribution to total variance of the stearoyl-CoA desaturase (*SCD1*), sterol regulatory element binding protein 1 (*SREBP-1*), and diacylglycerol acyltransferase 1 (*DGAT1*) genes and contribution to total variance of sire on milk fatty acids (FA) composition

Gene	Variable	μ	V(p)	Contribution to total variance of gene, %	Contribution to total variance of sire, %	Average gene substitution ¹
<i>SCD1</i>	C14:1 <i>cis</i> -9	0.871	0.062	4.350	20.039	0.052
	DI 14 ²	0.075	<0.001	8.944	9.044	0.004
<i>SREBP-1</i>	C15:0	0.929	0.045	2.043	8.648	0.069
	Odd-chain FA	2.091	0.120	1.930	7.234	0.136
<i>DGAT1</i>	C10:0	2.651	0.271	0.687	8.397	
	C14:1 <i>cis</i> -9	0.870	0.063	0.225	25.973	
	C16:0 <i>iso</i>	0.218	0.003	1.069	0.768	
	C16:1 <i>cis</i> -9	1.110	0.089	1.244	3.704	
	C18:0	8.615	2.588	0.881	0.228	
	C18:1 <i>trans</i> -6-8	0.195	0.001	2.518	3.456	
	C18:1 <i>trans</i> -10	0.414	0.052	0.742	0.552	
	C20:0	0.094	0.001	0.64	1.541	
	C18:2 <i>cis</i> -9 <i>trans</i> -11	0.467	0.010	0.433	2.239	
	C24:0	0.023	<0.001	0.465	2.325	
	DI 14	0.074	<0.001	0.943	20.878	
	DI 16 ³	0.040	0.001	0.666	3.346	
	DI 18 ⁴	0.676	0.002	1.309	4.420	
	RA/VA ⁵	0.342	0.002	1.266	2.153	

¹Average gene substitution was calculated only for *SCD1* and *SREBP-1*, because for *DGAT1* we used only AA and AK genotypes.

²DI (desaturation index) 14 = C14:1 *cis*-9/C14:0 + C14:1 *cis*-9.

³DI 16 = C16:1 *cis*-9/C16:0 + C16:1 *cis*-9.

⁴DI 18 = C18:1 *cis*-9/C18:0 + C18:1 *cis*-9.

⁵RA/VA = rumenic acid/vaccenic acid; C18:2 *cis*-9 *trans*-11/C18:1 *trans*-11 + C18:2 *cis*-9 *trans*-11.

other hand, this study is based on a smaller number of animals compared with Schennink et al. (2008; 351 vs. 1,779). Moreover, only 11 AK cows and 2 KK cows were available in this study, so our results need further investigation. However, these results confirm that *DGAT1* could be a candidate gene to explain differences in milk FA content.

Sire effects ranged from 0.23 to 25.97% of total variance (Table 6), showing an appreciable additive genetic effect on the variation in milk FA composition, as reported by Mele et al. (2007). The proportion of total variance explained by C14:1 *cis*-9 or DI 14 was higher than 20% for all genes considered.

CONCLUSIONS

In the present study, novel information about polymorphisms of 3 key genes involved in lipid metabolism was reported. The Italian Brown cattle showed an unbalanced allele distribution of the 3 genes, being the allele with the higher gene frequency present at 82, 84, and 98% for *SCD1*, *SREBP-1*, and *DGAT1*, respectively. The *SCD1* and *DGAT1* polymorphisms showed a significant association with milk FA composition, whereas the *SREBP-1* polymorphism did not seem significantly associated with milk FA composition. However, the unbalanced distribution of alleles in the

genes investigated suggests that the size of population should be increased.

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