Short communication: Effects of β -lactoglobulin, stearoyl-coenzyme A desaturase 1, and sterol regulatory element binding protein gene allelic variants on milk production, composition, acidity, and coagulation properties of Brown Swiss cows¹

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ABSTRACT

Associations of allelic variants of the β -lactoglobulin (*LGB*), stearoyl-coenzyme A desaturase 1 (*SCD*), and sterol regulatory element binding protein (*SREBP-1*) genes with milk production, composition (fat, protein, and casein content), acidity (pH and titratable acidity), and coagulation properties (rennet coagulation time and curd firmness) were investigated in Brown Swiss cows. In total, 294 animals (progeny of 15 sires) reared in 16 herds were milk-sampled once. The additive effects of *LGB* (rs109625649: C > T), *SCD* (ss469414220: C > T), and *SREBP-1* (AB355704: g.101_185ins) polymorphisms on the aforementioned traits were analyzed through Bayesian linear models. The *LGB* genotype affected rennet coagulation time, with TT (or BB) alleles showing longer rennet coagulation time compared with CC (or AA) cows. The *SCD* gene allelic variants were found to be associated with protein and casein contents and curd firmness: CC animals had the lowest values for the aforementioned traits. An association was found between *SREBP-1* alleles and fat content, with the highest values for cows carrying the 84-bp insertion (or L) allele. Results suggest a possible use of these loci in gene-assisted selection programs for the improvement of milk quality traits and coagulation properties in Brown Swiss cattle.

Key words: β -lactoglobulin (*LGB*), stearoyl-coenzyme A desaturase (*SCD*), sterol regulatory element binding protein (*SREBP-1*), milk coagulation property

Short Communication

The fraction of milk used for cheese making is growing worldwide. Under this scenario, milk coagulation properties (**MCP**), commonly measured as rennet coagulation time (**RCT**, min) and curd firmness (**a₃₀**, mm), become relevant for the dairy industry. Although genetic variation for these traits has been reported in several studies (Ikonen et al., 2004; Cassandro et al., 2008; Cecchinato et al., 2011), their inclusion as breeding goals in conventional selection programs is hampered by phenotyping costs. A possible solution can be found in the identification of candidate genes in addition to the frequently studied casein genes (e.g., Davoli et al., 1990; Comin et al., 2005, 2008; Penasa et al., 2010) that affect milk quality and MCP and that can be integrated in gene-assisted selection programs. Among the genes known to affect dairy traits, β -lactoglobulin (*LGB*) has been widely investigated for its relationships with MCP (Pagnacco and Caroli, 1987; Ikonen et al., 1999; Bonfatti et al., 2010), milk yield (Kuss et al., 2003), and milk composition (Heck et al., 2009). Another gene, stearoyl-coenzyme A desaturase 1 (*SCD*), has been found to be associated with milk fatty acid profile (Mele et al., 2007; Moioli et al., 2007; Kgwatalala et al., 2009) and milk and protein yields (Macciotta et al., 2008), but no investigation has been carried out for a possible association with MCP. Finally, another potential candidate gene is sterol regulatory element binding protein (*SREBP-1*); the *SREBP-1* locus has been proposed to play a central role in the regulation of milk fat synthesis (Harvatine and Bauman, 2006), controlling the expression of more than 30 genes (McPherson and Gauthier, 2004). Significant associations between *SREBP-1* allelic variants and fatty acid composition have been detected in subcutaneous fat (Hoashi et al., 2007), whereas the importance of *SREBP-1* allelic variants for dairy traits has yet to

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be assessed. The aim of this study was to investigate the association between *LGB*, *SCD*, and *SREBP-1* gene variants and milk yield, milk composition, and MCP in individual milk samples from Italian Brown Swiss cows.

Data were from 2 previous studies aimed at evaluating the reliability of MCP predicted by mid-infrared spectroscopy to be used as phenotypes in breeding programs for the improvement of MCP (Cecchinato et al., 2009; De Marchi et al., 2009), and for studying the associations of *SCD*, *DGAT1*, and *SREBP-1* variants with milk fatty acid composition (Conte et al., 2010). Briefly, 294 Brown Swiss cows (daughters of 15 AI sires) were sampled once from June 2006 to July 2007. Sampling was conducted in 16 herds 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 north-eastern Italy. Blood samples were collected in EDTA Vacutainers (BD Vacutainer Systems, Plymouth, UK) and stored at -20°C . After collection, without addition of preservative, milk samples were stored in a portable refrigerator (4°C) and transferred to the milk quality laboratory of the Veneto Agricoltura Institute (Thiene, Italy). Individual 10-mL milk samples were used for measuring MCP by a computerized renneting meter (Polo Trade, Monselice, Italy) within 3 h of sampling. Milk coagulation properties were measured for 31 min after the addition of rennet. Milk samples not forming a curd within 31 min were classified as noncoagulating. In addition to MCP, measures of milk yield, fat, protein, and casein content, pH, and titratable acidity were available. The extraction of DNA from blood was performed using the GFX Genomic Blood DNA Purification kit (Amersham Bioscience, Piscataway, NJ) as described by Conte et al. (2010). The *LGB* and *SCD* genotypes were determined by ligation detection reaction-universal array (Chessa et al., 2007; Conte et al.,

2010). This technique requires 2 discriminating probes (different fluorescent dye in 5') each carrying a different nucleotide in 3' (the 2 bases of the SNP) and a common probe that starts immediately after the SNP. The SNP investigated were *LGB* rs109625649:C > T (the C and the T alleles are also known as A and B alleles) and *SCD* ss469414220:C > T. The *SREBP-1* deletion of 84 bp (AB355705:g.101_185del), also known as S allele, and insertion of 84 bp (AB355704:g.101_185ins), also known as L allele, were analyzed by PCR (Conte et al., 2010), and the genotypes determined by 2% agarose gel. Some samples were sequenced by Primm Company (Milan, Italy) to confirm the genotype analyzed.

Calculations of allele frequencies and tests of Hardy-Weinberg equilibrium were carried out using Genepop software (Raymond and Rousset, 1995). An association study for the *LGB*, *SCD*, and *SREBP-1* gene variants was performed using Bayesian methodology. Milk production, quality, and MCP were analyzed from 294 records. The model included effects of DIM class (6 levels), parity (first, second, third, and greater than third), and the effect of *LGB*, *SCD*, and *SREBP-1* genotypes (with 3 levels each: CC, CT, TT for *LGB*; CC, CT, TT for *SCD*; and LL, LS, SS for *SREBP-1*). The model also included the effects of additive sire (**u**), herd (**h**), and residual (**e**). Note that test-day effects were confounded with herd effects, because all cows in a given herd were sampled on the same day, with sampling days differing among herds. Prior distributions for the additive genetic effects in **u** and herd effects in **h** were normal densities: $p(\mathbf{u} | \sigma_u^2) \sim N(0, \mathbf{A}\sigma_u^2)$, $p(\mathbf{h} | \sigma_h^2) \sim N(0, \mathbf{I}\sigma_h^2)$, where **A** was the numerator relationship matrix among sires, **I** was the identity matrix, and σ_u^2 and σ_h^2 were additive genetic and herd variances, respectively. Flat priors were used for systematic effects and dispersion parameters. Marginal posterior distributions of all parameters were obtained using the Gibbs sampler (Gelfand and Smith, 1990). In the present work, the Gibbs

Table 1. Descriptive statistics of test-day milk yield, composition, and coagulation properties for Brown Swiss cows ($n = 294$)

Trait ¹	Mean	SD	Minimum	Maximum
Milk yield, kg/d	30.32	7.03	14.3	54.6
Fat, %	3.88	0.74	1.48	6.58
Protein, %	3.67	0.35	2.75	4.59
Casein, %	2.86	0.27	2.26	3.56
pH	6.68	0.08	6.46	6.90
Titratable acidity, SH°/50 mL	3.27	0.39	1.40	4.54
RCT, min	15.52	4.19	8.20	30.00 ²
a ₃₀ , mm	40.52	9.40	8.00	64.00

¹SH° = Soxhlet-Henkel degrees; RCT = rennet coagulation time; a₃₀ = curd firmness for milk samples that coagulated.

²The maximum time allowed for curd formation.

sampler ran with a single chain of 100,000 points, and the first 10,000 were discarded as burn-in, previously tested by the Raftery and Lewis (1992) methodology. Our Bayesian approach considered the marginal posterior distribution of half of the difference between the estimated effects of homozygous genotypes (i.e., the additive effect). The posterior median was used as point estimate of parameters of concern. Lower and upper bounds of the 95% highest posterior probability density regions for additive and dominance effects were estimated from the Gibbs samples. In this case, the posterior probability (*P*) was the probability of a difference being greater than zero for positive effects or lower than zero for negative effects; from here, we considered an effect as relevant when the posterior probability over (or below) zero was >0.90.

Descriptive statistics for the investigated traits are reported in Table 1. A comprehensive discussion on these traits has been reported by Cecchinato et al. (2009), whereas genotype and allele frequencies of *SCD* and *SREBP-1* loci can be found in Conte et al. (2010). Regarding *LGB*, frequencies for T and C were 68 and 32%, respectively. Features of the estimated marginal posterior densities of additive effects for *LGB*, *SCD*, and *SREBP-1* alleles are presented in Table 2. All Monte Carlo standard errors were very small, and a lack of convergence was not detected by the Geweke test (data not shown; Geweke, 1992). Marginal posterior distributions were approximately normal; thus, mode, mean, and median were similar, and only the posterior median of the difference is shown.

The estimated additive effect of the *LGB* allelic variants was positive for RCT (0.67 min; *P* = 0.94) in agreement with Ikonen et al. (1999) and Bonfatti et al. (2010), indicating that the T allele was unfavorably associated with RCT (Table 2). Previous studies have reported contradictory effects of *LGB* genotypes; some authors detected a positive effect of the TT genotype (Lodes et al., 1996; Kübarsepp et al., 2005), whereas others detected a positive effect of the CC genotype (van den Berg et al., 1992; Ikonen et al., 1999). Others reported no effect (Pagnacco and Caroli, 1987; Ikonen et al., 1997; Hallén et al., 2007). It has been suggested (Lundén et al., 1997) that the favorable effect of the T allele on RCT detected in some studies might be ascribed to the increased total casein content associated with *LGB* T allele. Nevertheless, inconsistent results have been obtained for the association between RCT and protein and casein content. Ikonen et al. (2004) reported weak unfavorable or no genetic correlations between RCT and protein or casein content, whereas the opposite associations were assessed by Lindström et al. (1984) and Ikonen et al. (1999). Except for RCT, the additive effects for other traits were negligible.

Table 2. Features of the estimated marginal posterior densities of additive effects¹ for β-lactoglobulin (*LGB*), stearoyl-coenzyme A desaturase 1 (*SCD*), and sterol regulatory element binding protein (*SREBP-1*) gene variants²

Trait	<i>LGB</i> (T vs. C)			<i>SCD</i> (C vs. T)			<i>SREBP-1</i> (L vs. S)		
	Median	HPD 95%	<i>P</i>	Median	HPD 95%	<i>P</i>	Median	HPD95%	<i>P</i>
Milk yield, kg/d	-0.283	-1.381; 0.810	0.69	0.096	-1.460; 1.650	0.55	-0.076	-1.620; 1.460	0.54
Fat, %	0.060	-0.090; 0.211	0.78	-0.040	-0.245; 0.165	0.65	0.270	0.063; 0.477	0.99
Protein, %	-0.014	-0.065; 0.037	0.70	-0.089	-0.159; -0.0195	0.99	0.000	-0.068; 0.069	0.50
Casein, %	0.007	-0.034; 0.050	0.63	-0.066	-0.124; -0.008	0.99	0.000	-0.056; 0.057	0.50
pH	-0.004	-0.017; 0.009	0.72	0.003	-0.015; 0.022	0.63	-0.007	-0.025; 0.011	0.77
Titrate acidity, SH ⁺ /50 mL	-0.019	-0.09; 0.055	0.69	-0.097	-0.197; 0.002	0.97	0.032	-0.069; 0.133	0.73
RCT, min	0.670	-0.151; 1.497	0.94	-0.386	-1.536; 0.765	0.74	0.369	-0.769; 1.506	0.73
a ₉₀ , mm	0.420	-1.419; 2.269	0.67	-2.102	-4.816; 0.617	0.93	0.370	-2.196; 2.938	0.61

¹Additive effects were computed as half of the difference between the estimated effects of homozygous genotypes.
²Median = median of the marginal posterior density; HPD 95% = highest 95% posterior density interval; *P* = posterior probability of the differences being >0 for positive effects or <0 for negative effects (*P* >0.90 in bold).

The association between the *SCD* genotype and milk traits (Table 2) was significant for protein and casein content, titratable acidity, and a_{30} , with *P* varying from 0.93 to 0.99. In this study, the C allele generally showed unfavorable effects on MCP, because it reduced the titratable acidity and a_{30} . Similarly, in Canadian Jersey cows, the CC genotype was associated with lower 305-d protein and milk yields (Kgwatalala et al., 2009). It is worth noting that in a previous study carried out on Italian Holsteins, the CC genotype was found to be associated with higher milk and protein yield (Macciotta et al., 2008). Because previous studies identified some QTL for protein yield on the same chromosome as *SCD* (BTA 26) and close to the *SCD* gene (Plante et al., 2001; Boichard et al., 2003), the contrasting effects across dairy breeds of *SCD* allelic variants on protein yield could be due to causal mutations in the chromosome region, which harbors QTL for protein yield and *SCD* gene.

The L allele of the *SREBP-1* gene was associated with an increased fat content of 0.27% compared with the S allele (Table 2). According to Bionaz and Loor (2008), *SREBP-1* plays a pivotal role in milk fat synthesis in the cow through a complex metabolic pathway that involves several genes such as SREBP cleavage activating protein and insulin-induced gene 1 and 2. Because the decreased mammary expression of *SREBP-1* protein has been associated with a decrease of fat content (Harvatine and Bauman, 2006), more studies are needed to assess whether the effect of the investigated *SREBP-1* allelic variants on fat content is related to variation in mRNA and *SREBP-1* protein expression.

In conclusion, although further research is needed to investigate the effect of these genes on cheese yield, results support that changes in allele frequency at the *LGB*, *SCD*, and *SREBP-1* loci exert some effects on milk quality and MCP. Therefore, the association between these genes and milk traits could be exploited in gene-assisted selection programs for genetic improvement purposes. However, large-scale studies are required to confirm the effects found in the present work.

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