Effects of Stearoyl-CoA Desaturase 1 and Sterol Regulatory Element Binding Protein Gene Polymorphisms on Milk Production, Composition and Coagulation Properties of Individual Milk of Brown Swiss Cows

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Summary

Associations between stearoyl-CoA desaturase (*SCD*) and sterol regulatory element binding protein (*SREBP-1*) gene polymorphisms and milk production, composition (fat, protein, and casein content), acidity (pH and titratable acidity) and coagulation properties (MCP), namely rennet coagulation time (RCT, min) and curd firmness (a_{30}, mm) were investigated on individual Brown Swiss milk. A total of 294 cows from 16 herds and progeny of 15 sires were milk-sampled once. The additive effects of *SCD* and *SREBP-1* genotypes on the aforementioned traits were analyzed through Bayesian linear models. The *SCD* gene was associated with protein content, casein content and a_{30}. Lower protein, casein and a_{30} was observed for milk yielded by *SCD* V than A cows, whereas for other traits the effect was trivial. Animals carrying the L allele of *SREBP-1* showed higher fat content than animals carrying the S allele. These results suggest a possible use of these loci in gene-assisted selection programs for the improvement of milk quality traits and MCP in Brown Swiss cattle, although large scale studies in different breeds are required.

Key words

stearoyl-CoA desaturase (*SCD*), sterol regulatory element binding protein (*SREBP-1*), coagulation property, milk production traits

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Received: May 24, 2011 | Accepted: July 7, 2011

ACKNOWLEDGEMENTS
The authors wish to acknowledge the Italian Ministry of University and Research for financial support (PRIN2005-prot. 2005074889_002). The authors gratefully acknowledge also the Provincial Breeders Associations of Treviso, Venezia, Vicenza, Trento and Padova, and the Italian Brown Swiss Cattle Breeders Association (ANARB) for having provided milk recording and pedigree data. The support by Trento province and Superbrown Consortium of Bolzano and Trento is also acknowledged.

Aim

Milk coagulation properties (MCP) are influenced by several factors including genetic polymorphisms of some genes, which determine milk quality and composition (e.g. protein, casein and fat). This is very important in some country, like Italy, where more than 70% of milk is used for cheese production. The aim of this study was to investigate the association between *SCD* and *SREBP-1* genes and milk production, composition and coagulation properties of Brown Swiss individual milk.

Material and methods

Data was from two studies aimed at evaluating the relevance of MCP predictions obtained by mid-infrared spectroscopy as indicator traits in breeding programs for enhanced MCP in Italian Brown Swiss (Cecchinato et al., 2009) and to study, on the same breed, the associations between *SCD*, *DGAT1* and *SREBP-1* and milk fatty acids composition (Conte et al., 2010). A total of 294 cows were sampled once from June 2006 to July 2007. Cows were daughters of 15 AI sires and were distributed in 16 herds located in the North of Italy. Blood samples were collected in EDTA vacutainers (BD Vacutainer Systems, Plymouth, UK) and stored at -20°C. After collection, individual 10-mL milk samples were stored in portable refrigerator (4°C), transferred to the milk quality lab of Veneto Agricoltura Institute (Thiene, Italy) and analyzed MCP by a computerized renneting meter (Polo Trade, Monselice, Italy) within three hours from sampling. Milk coagulation properties were measured for 31 min since the addition of rennet and samples not forming a curd within this testing time were classified as non-coagulating. In addition to MCP, measures of fat, protein, and casein content, titratable acidity, and SCC were available. Values of SCC were converted to SCS by logarithm transformation (SCS = 3 + $\log_2$(SCC/100,000)). DNA was extracted from blood by GFX Genomic Blood DNA Purification kit (Amersham Bioscience, Piscataway, NJ) as described by Conte et al. (2010). *SCD* genotypes were determined by ligation detection reaction-universal array (LDR-UA) and the primers used were previously tested on Brown Swiss by Chessa et al. (2007). This technique requires 2 discriminating probes (different fluorescent dye in 5') each carrying a different nucleotide in 3'(the two bases of the SNPs) and a common probe that starts immediately after SNP. To distinguish the deletion of 84-bp (S-type) or insertion of 84-bp (L-type) on *SREBP-1* a PCR was performed (Conte et al., 2010) and the genotype was determined by 2% agarose gel. PRIMM srl confirmed the genotype analyzed by sequencing. Allelic frequencies and Hardy-Weinberg equilibrium were tested using Genepop software version 4.0 (Rousset and Raymond, 2007). An association study for the *SCD* and *SREBP-1* genes was performed using Bayesian methodology. Milk production, quality and MCP were analyzed from a total of 294 records. The model included effects of DIM (six classes), parity (first, second, third and higher than third) and *SCD* and *SREBP-1* genes genotype (three levels each: AA, AV, VV for *SCD* and LL, LS, and SS for *SREBP-1*). Note that test day effects were confounded with herd effects because all cows of a given herd were sampled once and in the same day. The model also included the additive effect of sire (**u**), the herd effect (**h**) and the residual effect (**e**). 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, 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 sampler was run 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 (MD) was used as point estimate of parameters of concern. Lower and upper bounds of the 95% highest posterior probability density regions for additive effects were estimated from the Gibbs samples. In this case, the posterior probability (P) above or below zero, was the probability of a difference bigger or smaller than zero, respectively, given the data; from here, we considered an effect as relevant when the posterior probability over (or below) zero was greater than 0.90.

Results and discussion

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 discussion about genotype and allele frequencies of *SCD* and *SREBP-1* genes, can be found in Conte et al. (2010). Features of the estimated marginal posterior densities of additive effects for *SCD* and *SREBP-1* genes are presented in Table 2. All Monte Carlo SE were very small and a lack of convergence was not detected by the Geweke test (data not shown). Marginal posterior distributions were approximately normal; thus mode, mean and median were similar, and only the posterior median of the difference is shown. The association between *SCD* gene with milk production, composition, acidity and MCP was relevant for protein, casein, titratable acidity and a_{30} with P varying from 0.93 to 0.99.

In this study the *V* allele showed general negative effects on MCP because it reduced the amount of the aforementioned traits. Previous studies revealed *V* as minor allele in Brown Swiss and in other breeds (Milanesi et al., 2008; Taniguchi et al., 2004; Mele

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

Trait[1]	Mean	SD	Min	Max
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	1.4	4.54
RCT, min	15.52	4.19	8.2	30
a_{30}, mm	40.52	9.40	8	64

[1]SH°: Soxhlet-Henkel degrees; SCS: $\log_2$(SCC/100,000) + 3; RCT: rennet coagulation time; a_{30}: curd firmness for milk samples that coagulated.

Table 2. Features of the estimated marginal posterior densities of additive effects for *SCD1* and *SREBP-1* genes[1,2]

Trait	*SCD1 V vs. A*			*SREBP-1 L vs. S*		
	MD	HPD95%	*P*	MD	HPD95%	*P*
Milk Yield, kg/d	0.096	-1.46; 1.65	0.55	-0.076	-1.62; 1.46	0.54
Fat, %	-0.040	-0.245; 0.165	0.65	0.270	0.063; 0.477	0.99
Protein, %	-0.089	-0.159; -0.0195	0.99	0.0005	-0.068; 0.069	0.50
Casein, %	-0.066	-0.124; -0.008	0.99	0.0005	-0.056; 0.057	0.50
pH	0.003	-0.015; 0.022	0.63	-0.007	-0.025; 0.011	0.77
Titratable acidity, SH°/50ml	-0.097	-0.197; 0.002	0.97	0.032	-0.069; 0.133	0.73
RCT, min	-0.386	-1.536; 0.765	0.74	0.369	-0.769; 1.506	0.73
a_{30}, mm	-2.102	-4.816; 0.617	0.93	0.370	-2.196; 2.938	0.61

[1]Additive deviations were computed as half the difference between the estimated effects of homozygous genotypes; [2]MD: median of the marginal posterior density; HPD95%: highest 95% posterior density interval; *P*: posterior probability of the differences being greater than 0 for positive effects or lower than 0 for negative effects.

et al., 2007) but in Piemontese breed the *V* showed higher frequency compared to *A* allele (Moioli et al., 2007). In this study the *V* allele showed general negative effects on MCP. With respect to the SREBP-1 gene, the *L* allele showed relevant effect, compared to *S*, increasing fat content of 0.27. According to Hoashi et al. (2007) this polymorphism contributes to fat composition and characteristics regulating gene transcription and activation even if Conte et al. (2010) reported a lack of association between *SREBP-1* and fatty acid composition; in our study fat parameter was estimated with MIRS analysis and results may be different compared with gas chromatography analysis.

Conclusions

The *SCD* gene seems to be more implicated in the worsening of milk parameters, especially for protein and a_{30}. Finally, this study suggests a possible use of these loci in gene-assisted selection programs for the improvement of milk quality traits, cheese making and MCP in Brown Swiss cattle, although large scale studies in different breeds are required.

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