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Genome-wide association of coagulation properties, curd firmness modeling, protein percentage, and acidity in milk from Brown Swiss cows

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ABSTRACT

Cheese production is increasing in many countries, and a desire toward genetic selection for milk coagulation properties in dairy cattle breeding exists. However, measurements of individual cheesemaking properties are hampered by high costs and labor, whereas traditional single-point milk coagulation properties (MCP) are sometimes criticized. Nevertheless, new modeling of the entire curd firmness and syneresis process (CF_t equation) offers new insight into the cheesemaking process. Moreover, identification of genomic regions regulating milk cheesemaking properties might enhance direct selection of individuals in breeding programs based on cheese ability rather than related milk components. Therefore, the objective of this study was to perform genome-wide association studies to identify genomic regions linked to traditional MCP and new CF_t parameters, milk acidity (pH), and milk protein percentage. Milk and DNA samples from 1,043 Italian Brown Swiss cows were used. Milk pH and 3 MCP traits were grouped together to represent the MCP set. Four CF_t equation parameters, 2 derived traits, and protein percentage were considered as the second group of traits (CF_t set). Animals were genotyped with the Illumina SNP50 BeadChip v.2 (Illumina Inc., San Diego, CA). Multitrait animal models were used to estimate variance components. For genome-wide association studies, the genome-wide association using mixed model and regression-genomic control approach was used. In total, 106 significant marker traits associations and 66 single nucleotide polymorphisms were identified on 12 chromosomes (1, 6, 9, 11, 13, 15, 16, 19, 20, 23, 26, and 28). Sharp peaks were detected at 84 to 88 Mbp on *Bos taurus* autosome (BTA) 6, with a peak at 87.4 Mbp in the region harboring the casein genes. Evidence of quantitative trait loci at 82.6 and 88.4 Mbp on the same chromosome was found. All chromosomes but BTA6, BTA11, and BTA28 were associated with only one trait. Only BTA6 was in common between MCP and CF_t sets. The new CF_t traits reinforced the support of MCP signals and provided with additional information on genomic regions that might be involved in regulation of the coagulation process of bovine milk.
Key words: genome-wide association study, milk coagulation, curd firmness, dairy cattle

INTRODUCTION

Milk composition (e.g., fat and protein content) as well as other milk features, such as its acidity (pH), are considered as the base for cheese manufacturing (Walstra et al., 2014). Moreover, cheese processing strongly depends on milk coagulation (clotting of milk by rennet enzymes) as well as the syneresis (shrinkage of the curd with expulsion of whey). Milk coagulation after rennet (or similar coagulation agents) addition is the first step to cheese production. Therefore, milk coagulation properties (**MCP**), such as rennet coagulation time (**RCT**, min), time to curd firmness of 20 mm ($\mathbf{k_{20}}$, min), and curd firmness 30 min after rennet addition ($\mathbf{a_{30}}$, mm), are important factors for the description of cheese manufacture. In addition, previous analyses have shown important genetic variation of the MCP traits [for a recent review on MCP genetics see Bittante et al. (2012)]. The heritability of MCP is higher compared with milk yield and similar to other quality traits of milk.

To overcome the problems related to the classical single-point estimates of MCP, such as late and noncoagulating milk samples and low repeatability, it has been proposed to model the curd firmness (**CF**) as a function of time (Bittante, 2011; Bittante et al., 2013). In this way, CF values are estimated over a longer time period through a model equation, thus providing extra information of the coagulation and CF processes (which

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also takes into account the phenomenon of syneresis). The wide scale recording of MCP values required for application in breeding programs adds difficulty because the phenotyping of MCP is highly costly and labor demanding. For wide application (at population level) of MCP, infrared spectroscopy has been promising (Cipolat-Gotet et al., 2012; Cecchinato et al., 2013; Chessa et al., 2014). An alternative is the identification of genomic regions regulating the aforementioned traits, linking the desired traits to the genome, which in turn may enhance establishment of marker-assisted selection programs or breeding programs based on whole-genome predictions (Van Eenennaam et al., 2014). For this purpose an experimental trial using daughter design and selective genotyping identified significant associations on chromosomes 2, 18, and 24 using coagulation as a binary trait (i.e., coagulating vs. noncoagulating milk in Finnish Ayrshire cattle; Tyrisevä et al., 2008). Significant associations of *LGB*, *CSN2*, and *GH1* with RCT have already been reported in candidate gene studies (Bonfatti et al., 2010; Cecchinato et al., 2015b). Moreover, pH has been associated with *GRLF1*, *LIPE*, and *SCD-1* (Cecchinato et al., 2015b). Cheese yield as well as MCP have also been associated with *LEP*, *LEPR*, and *CSN3* (Glantz et al., 2011). Recently, the CF and syneresis traits have also been tested for genomic associations, resulting in new candidate genes regulating cheesemaking properties of the milk in addition to those identified by the traditional MCP measures (Cecchinato et al., 2015b). Note, however, that all the previous studies were performed on a small number of preselected DNA markers and not on a whole-genome scale. With genome-wide association studies (**GWAS**), where a large number of SNP distributed along the whole genome are used, new, previously unknown, chromosomal regions associated with traits under investigation can potentially be identified (Schopen et al., 2011).

Exploration of the genetic background and identification of genomic regions affecting milk coagulation and CF might be useful for establishing gene-assisted selection programs or incorporate new knowledge for direct genomic prediction purposes (Glantz et al., 2012). A first attempt on GWAS for MCP traits has been recently presented using a high-density SNP chip but a relatively small number of individuals (379 cows; Gregersen et al., 2015). The aim of the present study was to apply GWAS on Italian Brown Swiss dairy cows genotyped with a 50k SNP chip. Traits investigated were traditional single-point MCP observations in connection to CF and syneresis traits in an effort to shed more light in the genomic background of cheesemaking-related traits. Milk acidity and protein percentage were also considered.

MATERIALS AND METHODS

Field Data

Milk samples from 1,264 Italian Brown Swiss cows were collected from 85 herds located in Trento Province in the northeast of Italy. With few exceptions, 15 cows from each herd were individually sampled once during evening milking. After collection, milk samples (without preservative) were immediately refrigerated (4°C). One random subsample was transported to the Milk Quality Laboratory of the Breeders Association of Trento Province (Trento, Italy) for composition analysis. The other subsample was transferred to the Cheese-Making Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Padova, Italy) for milk MCP analysis. All samples were processed within 20 h after collection. Information on cows and herds were provided by the Breeders Association of Trento Province (Italy). Phenotypic data were matched to pedigree information supplied by the Italian Brown Swiss Cattle Breeders Association (ANARB, Verona, Italy).

Analysis of Milk Quality and MCP

Individual milk subsamples were analyzed for fat, protein, and casein contents using MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark). The pH of the subsamples was measured before MCP analysis, using a Crison Basic 25 electrode (Crison, Barcelona, Spain).

Measures of MCP were obtained using the Formagraph instrument (**FRM**) by Foss Electric A/S according to the procedure described in Cipolat-Gotet et al. (2012). In brief, milk samples (10 mL) were heated to 35°C and 200 μL of a rennet solution (Hansen Standard 160, with 80 ± 5% chymosin and 20 ± 5% pepsin; 160 international milk clotting units/mL; Pacovis Amrein AG, Bern, Switzerland), diluted to 1.6% (wt/vol) in distilled water, was added at the beginning of analysis. Ten samples were analyzed simultaneously, one sample for each measuring unit of the coagulation meter (pendula), which records the width (mm) of the graph during testing every 15 s. The observation period continued for 90 min after rennet addition. Rennet coagulation time is defined as the time (min) from addition of enzyme to the beginning of coagulation, k_{20} (min) is the interval from RCT to the time at which a curd firmness of 20 mm is attained, and a_{30} (mm) is a measure of the extent of curd firmness 30 min after coagulant addition. Samples that did not coagulate within 30 min were classified as noncoagulating (Ikonen et al., 1999),