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# Association of candidate gene polymorphisms with milk technological traits, yield, composition, and somatic cell score in Italian Holstein-Friesian sires

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# ABSTRACT

Advances in DNA-based marker technology have enabled the identification of genomic regions underlying complex phenotypic traits in livestock species. The incorporation of detected quantitative trait loci into genetic evaluation provides great potential to enhance selection accuracies, hence expediting the genetic improvement of economically important traits. The objective of the present study was to investigate 96 single nucleotide polymorphisms (SNP) located in 53 candidate genes previously reported to have effects on milk production and quality traits in a population of highly selected Holstein-Friesian bulls. A total of 423 semen samples were used to genotype the bulls through a custom oligo pool assay. Forty-five SNP in 32 genes were found to be associated with at least 1 of the tested traits. Most significant and favorable SNP trait associations were observed for polymorphisms located in CCL3 and AGPAT6 genes for fat yield (0.037 and 0.033) kg/d, respectively), *DGKG* gene for milk yield (0.698) kg/d), PPARGC1A, CSN1S1, and AGPAT6 genes for fat percentage (0.127, 0.113, and 0.093%, respectively), GHR gene for protein (0.064%) and case percentage (0.053%), and TLR4 gene for fat (0.090%), protein (0.066%), and casein percentage (0.050%). Somatic cell score was favorably affected by GHR (-0.095) and POU1F1 (-0.137), and interesting SNP-trait associations were observed for polymorphisms located in CSN2, POU1F1, and AGPAT6 genes for rennet coagulation time (-0.592, -0.558, and -0.462 min,respectively), and GHR and CSN2 genes for curd firmness 30 min after rennet addition (1.264 and 1.183 mm, respectively). In addition to the influence of individual SNP, the effects of composite genotypes constructed by grouping SNP according to their individual effects on traits considered in the analysis were also examined. Favorable and significant effects on milk traits were observed for 2 composite genotypes, one including 10 SNP and the other 4 SNP. The former was associated with an increase of milk (0.075 kg/d), fat (0.097 kg/d), protein (0.083 kg/d), and casein yields (0.065 kg/d), and the latter was associated with an increase of fat (0.244%), protein (0.071%), and casein percentage (0.047%). Although further research is required to validate the identified SNP loci in other populations and breeds, our results can be considered as a preliminary foundation for further replication studies on gene-assisted selection programs.

**Key words:** candidate gene, milk coagulation trait, milk yield and composition, somatic cell count, Holstein bulls

#### INTRODUCTION

In recent decades, interest has been growing in the global quality and technological aspects of livestock products. In the dairy industry, contemporary breeding goals have broadened to include, along with milk production characteristics, health and functional traits in an effort to improve the overall functionality of the dairy cow. In many milk-producing countries, a large fraction of the milk is used for cheesemaking. In Italy, for example, more than 70% of the overall milk production is used to manufacture cheese; thus, milk technological traits are of great importance for the national dairy industry (Cassandro et al., 2008; Tiezzi et al., 2013). Important milk traits, which include milk yield, composition (fat, protein, and casein content), and milk coagulation properties (MCP), mainly described by rennet coagulation time (**RCT**; min) and curd firmness 30 min after rennet addition to milk  $(a_{30}; mm)$ , have therefore gained considerable interest worldwide. Milk coagulation properties have been reported to improve the efficiency of the cheesemaking process; in fact, milk with a high capacity to properly react to rennet and to produce a firm curd results in greater cheese yield (Bynum and Olson, 1982; Riddell-Lawrence and Hicks, 1989). Pretto et al. (2013) demonstrated that  $a_{30}$  has a positive effect on Grana Padano cheese yield, which

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in turn is expected to result in greater income for the dairy industry. The economic relevance of MCP has been recently reported by Cassandro et al. (2016), who estimated the economic values for RCT and  $a_{30}$  of milk destined to produce Grana Padano cheese in the Italian Holstein cattle population. Moreover, indirect selection for resistance to mastitis through the reduction of SCC is another important goal to be considered for improving milk production and quality.

Exploitable additive genetic variation for milk coagulation ability has been assessed in several cattle breeds in various countries. Heritability estimates of MCP are greater compared with milk yield and similar to other quality traits, ranging from 15 to 40% (Lindström et al., 1984; Ikonen et al., 2004; Penasa et al., 2010; Vallas et al., 2012). Moreover, the assessment of MCP can be routinely performed through mid-infrared spectroscopy, a technology widely used in milk recording programs to predict milk quality traits (Dal Zotto et al., 2008; De Marchi et al., 2008).

Traditionally, selection to improve profitability of livestock production has been based on EBV calculated from phenotypic records and pedigree, and on knowledge of the heritability of each trait (Goddard and Hayes, 2009). Nevertheless, several authors demonstrated the feasibility of improving cheesemaking-related traits through the identification of QTL affecting the traits (Ogorevc et al., 2009; Meredith et al., 2012; Cecchinato et al., 2014, 2015).

In recent years, due to their abundance and variability, SNP have been largely used in dairy cattle as powerful markers to identify loci underlying phenotypic variation in association studies. The candidate gene strategy allows focusing the analysis on particular genes involved in key metabolic pathways or physiological processes, which are probable to be involved in the traits of interest. The availability of many thousands of SNP has led to the development of genomic selection (Meuwissen et al., 2001). The advantage of this approach over traditional selection is that animals can be selected accurately early in life, based on their genomic predictions, and for traits that are difficult or expensive to measure. Currently, genomic selection is widely used in several countries, especially in the Holstein breed. Costs for analysis of high-density SNP genotyping have decreased dramatically in recent years, but they are still prohibitively high for expanding the analysis to the population level. The selection of a panel of few associated genes for a specific trait can be a viable strategy to reduce the cost of analysis for preselection and within-family selection of young bulls, especially for new traits for which national genetic evaluation is not yet performed, such as MCP. The objective of the present study was to evaluate the effect of 96 SNP and 3 composite genotypes within 53 candidate genes on milk production and composition traits, SCS, and MCP in Italian Holstein-Friesian sires.

# MATERIALS AND METHODS

### Sampling and Analysis of Milk Quality

From October 2011 to September 2014, a total of 292,007 individual milk samples from 45,115 Holstein-Friesian cows reared in Veneto Region (northeast Italy) and daughters of 4,531 sires, were collected during monthly test-day milk recording. Milk samples were collected according to the International Committee for Animal Recording (ICAR, 2009) guidelines and analyzed in the laboratory of the Breeders Association of Veneto region (Padova, Italy) using Milko-Scan FT6000 (Foss Electric A/S, Hillerød, Denmark). Traits recorded were fat  $(\mathbf{FP})$ , protein  $(\mathbf{PP})$ , and casein  $(\mathbf{CP})$ percentages, RCT, and a<sub>30</sub>. Mid-infrared spectroscopy models were implemented for routine prediction of MCP, as reported by De Marchi et al. (2013). In addition to quality traits and MCP, information on daily yields (kg/d) of milk (MY), fat (FY), protein (PY), and case in  $(\mathbf{CY})$  were available. Case in-to-protein  $(\mathbf{C}/\mathbf{P}; \%)$  and protein-to-fat  $(\mathbf{P}/\mathbf{F}; \%)$  ratios were also calculated. Values of SCC were determined with Cell Fossomatic 250 (Foss Electric A/S) and transformed to SCS to achieve normality and homogeneity of variances according to the formula of Wiggans and Shook (1987):  $SCS = 3 + \log_2(SCC/100,000).$ 

#### Sires DNA Extraction and Genotyping

Semen samples were collected from 423 sires and DNA extraction was carried out using the DNeasy Blood & Tissue Kit (catalog no. 69506, Qiagen, Valencia, CA), following the manufacturer's instructions. The extracted DNA was quantified with the Qubit System (Invitrogen, Carlsbad, CA) and assessed for integrity by 1% agarose gel electrophoresis.

Candidate gene selection was carried out using both a functional approach and a positional approach. In the functional approach, candidate genes were chosen on the basis of evidence of physiological or biochemical processes related to milk production and quality traits and involved in the immune system. For the positional approach, the identification of candidate genes was mainly based on the physical linkage information in chromosomal regions associated with milk composition and technological properties.

A first panel of 96 SNP was selected within 53 genes using either information available in the literature or in silico, after a database interrogation on NCBI dbSNP Download English Version:

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