J. Dairy Sci. 101:1–5
https://doi.org/10.3168/jds.2018-14533

Short communication: Association analysis of diacylglycerol acyltransferase (DGAT1) mutation on chromosome 14 for milk yield and composition traits, somatic cell score, and coagulation properties in Holstein bulls

T. Bobbo,* F. Tiezzi,† M. Penasa,*[1] M. De Marchi,* and M. Cassandro*
*Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, 35020 Legnaro, Padova, Italy
†Department of Animal Science, North Carolina State University, Raleigh 27695

ABSTRACT

The aim of the present study was to determine the allele frequencies of the diacylglycerol acyltransferase (DGAT1) K232A mutation in Italian Holstein bulls and to estimate the effect of the mutation on milk yield, composition, somatic cell score, and coagulation traits (rennet coagulation time and curd firmness). For this purpose, 349 Italian Holstein bulls were genotyped for the DGAT1 mutation on chromosome 14. Association analysis was performed by regressing the number of copies for the K allele on the deregressed estimated breeding value of the individual. Breeding values were calculated using field data routinely collected in Northeast Italy. The frequencies of the AA, KA, and KK genotypes were 59.6, 32.1, and 8.3%, respectively, and the minor allele frequency (K variant) was 24.7%. The K allele was significantly associated with greater fat yield and fat, protein, and casein percentages and with reduced protein:fat ratio. The association between the DGAT1 mutation and somatic cell score was not significant, whereas a favorable association between presence of the K allele and milk coagulation properties was found. Results from the present study confirmed the effect of the diallelic DGAT1 polymorphism K232A on milk production traits and, for the first time, provided evidence that this mutation also affects milk coagulation properties in the Italian Holstein breed.
Key words: coagulation trait, diacylglycerol acyltransferase, dairy cattle, milk composition

Short Communication

The use of genomic information for animal selection is a valuable tool in genetic improvement programs (Miglior et al., 2017). Association studies using a candidate-gene approach are common methods for exploring the relationship between allelic variation in specific relevant regions of the genome and the underlying phenotypes of interest. In recent decades, several genes affecting economically important traits in livestock species have been localized within QTL dispersed across the genome. In cattle breeds, the diacylglycerol acyltransferase (**DGAT1**), a candidate gene located on the centromeric region of *Bos taurus* autosome 14, encodes acyl coenzyme A:diacylglycerol acyltransferase, a protein involved in fat metabolism (Grisart et al., 2002; Winter et al., 2002). Polymorphisms in the DGAT1 gene, resulting from a lysine to alanine substitution at position 232 (K232A mutation), have been associated with differences in the kinetics of the enzymes encoded by the 2 allelic variants (Grisart et al., 2004). In particular, Grisart et al. (2004) demonstrated that the lysine variant, which represents the "wild type" and is defined as K allele, is characterized by a higher V_{max} (maximum rate of reaction) of the enzyme in synthesizing triglycerides compared with the alanine variant (A allele), thus increasing the fat percentage in the milk of the animal.

The distribution of the allele frequencies of the DGAT1 K232A mutation has been evaluated in different Holstein populations and other dairy cattle breeds, and the effects of the DAGT1 polymorphisms on milk production traits have been widely investigated (Gautier et al., 2007; Barbosa da Silva et al., 2010; Manga and Říha, 2011). In particular, the lysine variant has been associated with increased fat yield and fat and protein percentages, whereas the alanine variant has been associated with increased milk and protein yield (Spelman et al., 2002; Näslund et al., 2008). Nevertheless, the diallelic DGAT1 effect on milk production traits also could be partially explained by the presence of multiple alleles at the DGAT1 locus or other mutations in closely related genes (Bennewitz et al., 2004; Kühn et al., 2004). Furthermore, the DGAT1 gene may also affect nonproduction traits such as carcass fatness (Thaller et al., 2003), conformation, reproduction (Kaupe et al., 2007), body energy, and blood metabolic

Received February 2, 2018.
Accepted May 23, 2018.
[1] Corresponding author: mauro.penasa@unipd.it

traits (Oikonomou et al., 2009). However, the effect of DGAT1 on nonproduction traits is still controversial; in fact, Berry et al. (2010) reported no association between the K232A mutation and fertility, survival, calving performance, and conformation traits, with the only exception of rump width.

To date, the effect of DGAT1 mutation on milk quality and some nonproduction traits has been evaluated, whereas the effect of this gene on milk technological traits such as milk coagulation properties (**MCP**) has not yet been investigated. These traits are relevant in many countries because a large amount of the produced milk is used to manufacture cheese, and their effect on cheese yield and quality has been demonstrated (Pretto et al., 2013; Visentin et al., 2017). Therefore, the aim of the present study was to determine the allele frequencies of the DGAT1 K232A mutation in Italian Holstein bulls and to estimate the effect of the 2 allelic variants on milk yield, milk composition, SCS, and MCP.

For the present study, 349 Italian Holstein sires were genotyped for the DGAT1 mutation on chromosome 14 following the methodologies reported in Conte et al. (2010) and Viale et al. (2017). Briefly, semen samples were collected and DNA extraction was performed using the DNeasy blood and tissue kit (catalog no. 69506; Qiagen, Valencia, CA). The Qubit System (Invitrogen, Carlsbad, CA) was used for DNA quantification, and DNA integrity was assessed by 1% agarose gel electrophoresis. Genotyping was performed with the Illumina GoldenGate Assay (Illumina Inc., San Diego, CA), and the GeneCall software (Illumina) with a GCscore threshold of 0.25 was used for automatic allele calling.

Once genotype and allele frequencies were determined, a chi-squared test was used to examine whether the population deviated from Hardy-Weinberg equilibrium using the R package HardyWeinberg (Graffelman, 2015). Approximate standard errors of allele frequencies were calculated as in Banos et al. (2008) by the square root of $P(1 - P)/n$, where P = allelic frequency and n = number of bulls.

Association analysis was performed by regressing the number of copies for the K allele on the deregressed estimated breeding value (**dEBV**) of the individual. Breeding values were calculated from field data routinely collected in the Veneto region of Northeast Italy. Traits included milk yield; fat, protein, and casein percentages; SCC; and MCP [i.e., rennet coagulation time (**RCT**; min) and curd firmness 30 min after rennet addition ($\mathbf{a_{30}}$; mm)]. Fat yield, protein yield, casein yield, protein:fat ratio, and casein:protein ratio were derived from previous field traits. Mid-infrared spectroscopy calibration models for routine prediction of MCP (De Marchi et al., 2012) were installed on MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark) of the laboratory of the Breeders Association of Veneto Region (Padova, Italy). Coefficients of determination in cross-validation were 0.76 for RCT and 0.70 for a_{30} (De Marchi et al., 2012), suggesting high correlations between measured and predicted traits (0.87 and 0.83, respectively). Somatic cell count was log-transformed to SCS according to Wiggans and Shook (1987). Data editing and statistical model used for EBV calculation were the same as reported by Tiezzi et al. (2013) but applied to data collected from September 2011 to June 2017. After editing, 1,309,884 observations from 156,391 cows (daughters of 6,544 bulls) and 1,479 herds were available. The dEBV were obtained following Garrick et al. (2009), and only individuals with dEBV reliability greater than 0.20 for all traits considered were used in the analysis. The model used for association analysis was a single marker regression with the number of copies of the K allele as fixed effect:

$$y_{ij} = \mathbf{X}\mathbf{b}_i + \mathbf{Z}a_j + \frac{e_{ij}}{w_{ij}},$$

where y_{ij} is the pseudo-phenotype (dEBV) for the jth individual, $\mathbf{b}_i$ is a vector of solutions for the population mean and average allele substitution (fixed) effect, a_j is the animal additive polygenic effect, e_{ij} is the residual, w_{ij} is the weight of the y_{ij} dEBV, $\mathbf{X}$ is the incidence matrix reporting a vector of 1s and the number of copies of the K allele (0, 1, or 2 for the genotypes AA, AK, and KK, respectively), and $\mathbf{Z}$ is the incidence matrix for the animal additive polygenic effect.

Statistical analyses were implemented in a Bayesian framework using Gibbs sampling. For all models, priors for additive genetic effects a were multivariate normal $a \sim N\left(0, \mathbf{A}\sigma_a^2\right)$, where $\mathbf{A}$ is the numerator relationship matrix, whereas priors for residual $\left(\sigma_e^2\right)$ and additive polygenic variance $\left(\sigma_a^2\right)$ followed an inverted chi-squared distribution *invChisq*(ν, S), where ν is the degrees of freedom and S is the scale. Whereas ν was considered equal to 6 for all priors, S was chosen according to the expectation of variances for the specific trait. Expectations of additive polygenic and residual variance were inferred running a simplified model without marker effect using a noninformative prior. Therefore, given V_a and V_e as expectation of additive polygenic and residual variance, respectively, additive polygenic effect variance had scale $S_a = V_a(\nu - 2)$, whereas residual variance had scale $S_e = V_e(\nu - 2)$. Average allele substitution effect and population mean were sampled from a flat prior. Gibbs chains were run for 250,000 iterations with