

J. Dairy Sci. 93:3346–3349 doi:10.3168/jds.2010-3164 © American Dairy Science Association<sup>®</sup>, 2010. Open access under CC BY-NC-ND license.

# Short communication: Influence of composite casein genotypes on additive genetic variation of milk production traits and coagulation properties in Holstein-Friesian cows

**M. Penasa,\* M. Cassandro,\*<sup>1</sup> D. Pretto,\* M. De Marchi,\* A. Comin,\* S. Chessa,†<sup>2</sup> R. Dal Zotto,\* and G. Bittante\*** \*Department of Animal Science, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy †Department of Veterinary Science and Technology for Food Safety, University of Milano, Via Celoria 10, 20133 Milano, Italy

## ABSTRACT

The aim of the study was to quantify the effects of composite  $\beta$ - and  $\kappa$ -case (CN) genotypes on genetic variation of milk coagulation properties (MCP); milk yield; fat, protein, and CN contents; somatic cell score; pH; and titratable acidity (TA) in 1,042 Italian Holstein-Friesian cows. Milk coagulation properties were defined as rennet coagulation time (RCT) and curd firmness  $(a_{30})$ . Variance components were estimated using 2 animal models: model 1 included herd, days in milk, and parity as fixed effects and animal and residual as random effects, and model 2 was model 1 with the addition of composite  $\beta$ - and  $\kappa$ -CN genotype as a fixed effect. Genetic correlations between RCT and  $a_{30}$  and between these traits and milk production traits were obtained with bivariate analyses, based on the same models. The inclusion of casein genotypes led to a decrease of 47, 68, 18, and 23% in the genetic variance for RCT,  $a_{30}$ , pH, and TA, respectively, and less than 6% for other traits. Heritability of RCT and  $a_{30}$  decreased from 0.248 to 0.143 and from 0.123 to 0.043, respectively. A moderate reduction was found for pH and TA, whereas negligible changes were detected for other milk traits. Estimates of genetic correlations were comparable between the 2 models. Results show that composite  $\beta$ - and  $\kappa$ -CN genotypes are important for RCT and  $a_{30}$  but cannot replace the recording of MCP themselves.

**Key words:** additive variance, casein genotype, coagulation property, Holstein breed

The improvement of milk coagulation properties (MCP) is a hot topic in dairy cattle genetics, mainly because the amount of milk used for manufacturing cheese is growing worldwide (Schmit and Kaiser, 2006) and several studies have confirmed the role of MCP

in cheese-making (Aleandri et al., 1989; Wedholm et al., 2006; De Marchi et al., 2008). Exploitable additive genetic variation exists for coagulation ability of milk, and heritability estimates range from 15 to 40% (Ikonen et al., 1999a; Ikonen et al., 2004; Cassandro et al., 2008). Recently, De Marchi et al. (2009) proposed the use of mid-infrared spectroscopy to routinely record MCP on individual milk samples, and Cecchinato et al. (2009) suggested that the application of this technique on a large scale for the genetic improvement of MCP is feasible.

Casein polymorphisms have been extensively investigated in the past along with their associations with milk production traits (Ng-Kwai-Hang et al., 1986; Aleandri et al., 1990) and technological properties of milk (Okigbo et al., 1985; Marziali and Ng-Kwai-Hang, 1986). In these studies, attention was focused on the effect of individual milk CN loci separately rather than the effect of composite genotypes on yield, composition, and cheese-making properties of milk. Because of the close linkage between the CN loci on the bovine chromosome 6 (Ferretti et al., 1990; Threadgill and Womack, 1990), the alleles of different CN are in linkage disequilibrium, leading to biases in estimation of genotype effects when individual CN are simultaneously included in a model (Mayer et al., 1997; Ojala et al., 1997; Ikonen et al., 1999b). Consequently, the use of CN genotype combinations or haplotypes is a more appropriate approach to estimate the effects of these loci on milk production traits (Ikonen et al., 1999b, 2001; Boettcher et al., 2004; Caroli et al., 2004) and MCP (Hallén et al., 2007; Comin et al., 2008).

Only Ikonen et al. (1999a) investigated changes in additive genetic variance of MCP when records were adjusted for CN genotype effects using an animal model. That study was mainly based on information from Finnish Ayrshire cows, and no data are currently available on the effects of aggregate CN genotypes on the genetic variation of MCP in Holstein populations. Hence, the objective of the present study was to quantify the contribution of composite  $\beta$ - and  $\kappa$ -CN genotypes on additive genetic variance of rennet coagulation time

Received February 11, 2010.

Accepted March 29, 2010.

<sup>&</sup>lt;sup>1</sup>Corresponding author: martino.cassandro@unipd.it

<sup>&</sup>lt;sup>2</sup>Present address: Institute of Agricultural Biology and Biotechnology (IBBA), National Research Council, Via Bassini 15, 20133 Milano, Italy.

(RCT; min), curd firmness  $(a_{30}; mm)$ , milk yield, and milk quality traits in Italian Holstein-Friesian cows.

Data from a previous research study were used (for details see Cassandro et al., 2008). Briefly, a total of 1,042 multiparous Holstein cows, progeny of 54 AI sires, were sampled once in 34 herds located across the provinces of Padova, Treviso, and Venezia (northeast Italy) from January to July 2004. The average number of daughters per sire was 19 (range: 3–86), and the average number of sampled cows per herd was 31 (range: 8–107). Milk samples were collected during the morning milking concurrently with the monthly test-day milk recording and were analyzed within 3 h from collection for RCT; a<sub>30</sub>; milk yield; fat, protein, and CN contents; SCC; pH; and titratable acidity (TA, Soxhlet-Henkel degrees/50 mL). Values of SCC were transformed to SCS by means of a logarithmic function. Milk coagulation properties were measured through a computerized renneting meter (Polo Trade, Monselice, Italy) for 31 min after the addition of rennet. Samples not forming a curd within this testing time were classified as noncoagulating (9.7%) of the total) and were excluded from the subsequent statistical analysis of RCT and  $a_{30}$ . Isoelectric focusing analysis (Erhardt, 1989) was used to determine the CN genetic polymorphisms at  $\beta$ - and  $\kappa$ -CN loci. Descriptive statistics of all the variables are summarized in Cassandro et al. (2008), and observed frequencies of composite  $\beta$ - and  $\kappa$ -CN genotypes are reported in Comin et al. (2008).

Heritability for the studied traits was estimated using single-trait animal models, whereas genetic correlations between MCP and milk production traits were assessed using 14 sequential bivariate analyses in which a milk coagulation characteristic was analyzed simultaneously with milk yield, fat content, protein content, CN content, SCS, pH, or TA. The genetic correlation between RCT and  $a_{30}$  was also estimated in these analyses. All models accounted for herd (34 levels), DIM (14 levels: 10 monthly classes up to 300 d, 3 bimonthly classes up to 480 d, and 1 open class beyond 480 d), and parity (3 levels: first, second, and third and later lactations) as fixed effects and additive genetic animal and residual as random effects. To investigate the contribution of the composite  $\beta$ - and  $\kappa$ -CN genotypes on additive genetic variance of the traits, the aforementioned analyses were repeated including the composite genotypes grouped into 16 classes as described in Comin et al. (2008).

Variance and covariance components for the random factors were obtained with the VCE software package (Neumaier and Groeneveld, 1998), which uses REML procedures. The number of animals in the additive relationship matrix was 7,387 and included all cows with phenotypic record and their ancestors up to 8 generations back. Pedigree information was supplied by the Italian Holstein Friesian Cattle Breeders Association (ANAFI, Cremona, Italy).

The inclusion of CN genotypes led to a decrease of 47 and 68% in the genetic variance for RCT and  $a_{30}$ , respectively (Table 1), revealing the strong contribution of genotypes to MCP variation and suggesting that  $\beta$ - and  $\kappa$ -CN are major genes for RCT and  $a_{30}$ in Italian Holstein-Friesian cows. Results agree with the relevant effect of the genotypes on MCP in this population (Comin et al., 2008). Few research studies have reported the effect of milk protein genotypes on additive genetic variation of MCP (Oloffs et al., 1992; Ikonen et al., 1999a). In particular, Ikonen et al. (1999a) adjusted MCP records of Finnish dairy cows for composite  $\beta$ - and  $\kappa$ -CN and  $\beta$ -LG genotype effects and found a much less pronounced influence of them on estimates of genetic variance of RCT and  $a_{30}$  (20 and 24%, respectively) compared with the present study. On the other hand, Oloffs et al. (1992) reported an increase in additive genetic variation of MCP as milk protein genotypes were included in the model; this disagrees with the notable effect of protein loci on technological properties of milk. However, the authors did not use an animal model, and this could have influenced the outcome of their analysis.

The contribution of composite  $\beta$ - and  $\kappa$ -CN genotypes to additive genetic variance for milk yield; fat, protein, and CN contents; and SCS was negligible (Table 1), in accordance with findings from Ikonen et al. (1999a). Ojala et al. (1997) reported that composite  $\kappa$ - $\beta$ - $\alpha_{S1}$ -CN genotypes explained approximately 15 and 7% of the additive genetic variance for milk production and fat content, respectively, in Holstein-Friesian cows in California. The genetic variance of pH and TA was reduced by 18 and 23%, respectively, when records where adjusted for composite  $\beta$ - and  $\kappa$ -CN genotypes (Table 1). Ikonen et al. (1999a) reported a negligible effect of composite genotypes on pH, whereas no studies quantifying the effect on TA are available in the scientific literature that the authors are aware of.

As a consequence of the relevant changes in additive genetic variation, heritability estimate decreased from 0.248 to 0.143 for RCT and approached zero for  $a_{30}$ , changing from 0.123 to 0.043 (Table 1). For pH and TA, moderate changes were detected (0.216 to 0.181 and 0.195 to 0.154, respectively), whereas changes were negligible for other milk traits. Differences were not always significantly greater than zero (P > 0.05), but heritability estimates consistently decreased after including the genotype effects in the model (except for fat content). Furthermore, changes in RCT and  $a_{30}$  were considerable, suggesting that these loci may account for a substantial proportion of heritability. Ojala et al. (1997) observed that removing milk protein genotypes

#### PENASA ET AL.

Table 1. Variance components<sup>1</sup> and heritability of coagulation properties, milk yield, and milk quality traits obtained with 2 linear models<sup>2</sup>

	σ	2 e	$\sigma^2$	a		$h^2 \pm SE$		- 9
Trait	Model 1	Model 2	Model 1	Model 2	$\Delta \sigma_{a}^{2}, \%$ (model 2 - 1)	Model 1	Model 2	$\frac{h^2}{(\text{model } 1-2)}$
Rennet coagulation time, min	13.3423	13.9202	4.3992	2.3289	-47	$0.248 \pm 0.065$	$0.143 \pm 0.063$	0.105
Curd firmness, mm	92.7888	92.6136	13.0255	4.1247	-68	$0.123 \pm 0.049$	$0.043 \pm 0.042$	0.080
Milk yield, kg/d	41.3220	40.8556	3.1168	3.0046	-4	$0.070 \pm 0.040$	$0.069 \pm 0.041$	0.001
Fat, %	0.2649	0.2648	0.1790	0.1809	+1	$0.403 \pm 0.079$	$0.406 \pm 0.074$	-0.003
Protein, %	0.0647	0.0656	0.0293	0.0278	-5	$0.312 \pm 0.073$	$0.298 \pm 0.072$	0.014
CN, %	0.0408	0.0412	0.0214	0.0206	-4	$0.344 \pm 0.072$	$0.333 \pm 0.072$	0.011
SCS	3.0613	3.0675	0.1436	0.1423	-1	$0.045 \pm 0.030$	$0.044 \pm 0.030$	0.001
pH	0.00599	0.00616	0.00165	0.00136	-18	$0.216 \pm 0.060$	$0.181 \pm 0.064$	0.035
TA, <sup>3</sup> SH°/50 mL	0.1081	0.1119	0.0262	0.0203	-23	$0.195\pm0.060$	$0.154\pm0.062$	0.041

 ${}^{1}\sigma_{e}^{2}$  = residual variance;  $\sigma_{a}^{2}$  = additive genetic variance;  $h_{\beta-\kappa-CN}^{2}$  = contribution of the composite  $\beta$ - and  $\kappa$ -CN genotypes to  $h^{2}$  from model 1 (model 1 - 2).

 $^2Model~2$  is model 1 with the inclusion of composite  $\beta\text{-}$  and  $\kappa\text{-}CN$  genotypes.

<sup>3</sup>TA = titratable acidity; SH<sup>°</sup> = Soxhlet-Henkel degrees.

from the model increased heritability estimates of milk production and fat content by 0.05 and 0.03 units, respectively.

Genetic correlations between RCT and  $a_{30}$  and between them and milk production traits obtained by including composite  $\beta$ - and  $\kappa$ -CN information (model 2) were almost comparable with those obtained using model 1, but with higher standard errors of estimates (Table 2). No reason for the increase of standard errors was immediately obvious. Only the genetic correlation between SCS and MCP increased notably (more than doubled) in absolute value, albeit nonsignificantly (P > 0.05), when composite genotypes were included in the analysis. No estimates of the amount of changes in genetic correlations of MCP with production and quality traits after adjustment for composite genotypes are available in the literature, and further research is needed on a larger data set to confirm these results.

In conclusion, this study reported the notable influence of composite  $\beta$ - and  $\kappa$ -CN genotypes on MCP and the moderate to negligible effect on milk production traits. Heritability of RCT was still appreciable after adjustment for composite  $\beta$ - and  $\kappa$ -CN genotypes, suggesting that the recording of this trait cannot be replaced by genotyping of animals for milk protein variants. However, because MCP are affected by CN loci and because inexpensive and powerful techniques for simultaneous detection of the genetic variants are available, it can be argued that combining RCT data and CN information could provide a tool to better address future selection decisions aiming to improve MCP, particularly in countries such as Italy where the majority of milk is destined to cheese production.

### ACKNOWLEDGMENTS

The authors thank PRIN 2003 project (prot. 2003078003\_002 and 20030708003\_004) of the Italian Ministry of University and Research (MIUR) for funding the research, Emma Tealdo and the milk quality laboratory of Veneto Agricoltura (Thiene, Italy) for milk renneting analysis, the Breeders Association of

Table 2. Genetic correlations ( $\pm$ SE) between coagulation properties and milk yield and milk quality traits obtained with 2 linear models<sup>1</sup>

	Rennet coag	gulation time	Curd firmness		
Trait	Model 1	Model 2	Model 1	Model 2	
Rennet coagulation time, min			$-0.974 \pm 0.050$	$-0.960 \pm 0.108$	
Milk yield, kg/d	$-0.173 \pm 0.263$	$-0.355 \pm 0.343$	$0.186 \pm 0.314$	$0.272 \pm 0.476$	
Fat, %	$0.030 \pm 0.160$	$0.096 \pm 0.195$	$0.036 \pm 0.197$	$-0.033 \pm 0.278$	
Protein, %	$-0.037 \pm 0.175$	$-0.009 \pm 0.222$	$0.435 \pm 0.211$	$0.484 \pm 0.377$	
CN, %	$-0.154 \pm 0.169$	$-0.128 \pm 0.214$	$0.559 \pm 0.194$	$0.667 \pm 0.444$	
SCS	$0.168 \pm 0.321$	$0.538 \pm 0.348$	$-0.312 \pm 0.345$	$-0.700 \pm 0.447$	
pH	$0.851 \pm 0.093$	$0.862 \pm 0.126$	$-0.908 \pm 0.117$	$\mathrm{NE}^2$	
TA, <sup>3</sup> SH°/50 mL	$-0.502 \pm 0.167$	$-0.326 \pm 0.256$	$0.727 \pm 0.104$	$0.841 \pm 0.394$	

<sup>1</sup>Model 2 is model 1 with the inclusion of composite  $\beta$ - and  $\kappa$ -CN genotypes.

 $^{2}NE = not estimable.$ 

 ${}^{3}TA = titratable acidity; SH^{\circ} = Soxhlet-Henkel degrees.$ 

Journal of Dairy Science Vol. 93 No. 7, 2010

Padova, Treviso, and Venezia provinces, and the Italian Holstein Friesian Cattle Breeders Association (ANAFI, Cremona, Italy) for supplying milk recording data and pedigree information. The useful comments and suggestions provided by the section editor and 2 anonymous reviewers are gratefully acknowledged.

## REFERENCES

- Aleandri, R., L. G. Buttazzoni, J. C. Schneider, A. Caroli, and R. Davoli. 1990. The effects of milk protein polymorphisms on milk components and cheese-production ability. J. Dairy Sci. 73:241–255.
- Aleandri, R., J. C. Schneider, and L. G. Buttazzoni. 1989. Evaluation of milk for cheese production based on milk characteristics and Formagraph measures. J. Dairy Sci. 72:1967–1975.
- Boettcher, P. J., A. Caroli, A. Stella, S. Chessa, E. Budelli, F. Canavesi, S. Ghiroldi, and G. Pagnacco. 2004. Effects of casein haplotypes on milk production traits in Italian Holstein and Brown Swiss cattle. J. Dairy Sci. 87:4311–4317.
- Caroli, A., S. Chessa, P. Bolla, E. Budelli, and G. C. Gandini. 2004. Genetic structure of milk protein polymorphisms and effects on milk production traits in a local dairy cattle. J. Anim. Breed. Genet. 121:119–127.
- Cassandro, M., A. Comin, M. Ojala, R. Dal Zotto, M. De Marchi, L. Gallo, P. Carnier, and G. Bittante. 2008. Genetic parameters of milk coagulation properties and their relationships with milk yield and quality traits in Italian Holstein cows. J. Dairy Sci. 91:371–376.
- Cecchinato, A., M. De Marchi, L. Gallo, G. Bittante, and P. Carnier. 2009. Mid-infrared spectroscopy predictions as indicator traits in breeding programs for enhanced coagulation properties of milk. J. Dairy Sci. 92:5304–5313.
- Comin, A., M. Cassandro, S. Chessa, M. Ojala, R. Dal Zotto, M. De Marchi, P. Carnier, L. Gallo, G. Pagnacco, and G. Bittante. 2008. Effects of β- and κ-casein genotypes on milk coagulation, quality, and yield traits in Italian Holstein cows. J. Dairy Sci. 91:4022–4027.
- De Marchi, M., G. Bittante, R. Dal Zotto, C. Dalvit, and M. Cassandro. 2008. Effect of Holstein Friesian and Brown Swiss breeds on quality of milk and cheese. J. Dairy Sci. 91:4092–4102.
- De Marchi, M., C. C. Fagan, C. P. O'Donnell, A. Cecchinato, R. Dal Zotto, M. Cassandro, M. Penasa, and G. Bittante. 2009. Prediction of coagulation properties, titratable acidity, and pH of bovine milk using mid-infrared spectroscopy. J. Dairy Sci. 92:423–432.
- Erhardt, G. 1989. κ-Casein in bovine milk. Evidence of a further allele (κ-CnE) in different breeds. J. Anim. Breed. Genet. 106:225– 231.
- Ferretti, L., P. Leone, and V. Sgaramella. 1990. Long range restriction analysis of the bovine casein genes. Nucleic Acids Res. 18:6829– 6833.

- Hallén, E., T. Allmere, J. Näslund, A. Andrén, and A. Lundén. 2007. Effect of genetic polymorphism of milk proteins on rheology of chymosin-induced milk gels. Int. Dairy J. 17:791–799.
- Ikonen, T., K. Ahlfors, R. Kempe, M. Ojala, and O. Ruottinen. 1999a. Genetic parameters for the milk coagulation properties and prevalence of noncoagulating milk in Finnish dairy cows. J. Dairy Sci. 82:205–214.
- Ikonen, T., H. Bovenhuis, M. Ojala, O. Ruottinen, and M. Georges. 2001. Associations between casein haplotypes and first lactation milk production traits in Finnish Ayrshire cows. J. Dairy Sci. 84:507–514.
- Ikonen, T., S. Morri, A.-M. Tyrisevä, O. Ruottinen, and M. Ojala. 2004. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. J. Dairy Sci. 87:458–467.
- Ikonen, T., M. Ojala, and O. Ruottinen. 1999b. Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows. J. Dairy Sci. 82:1026–1033.
- Marziali, A. S., and K. F. Ng-Kwai-Hang. 1986. Effects of milk composition and genetic polymorphism on coagulation properties of milk. J. Dairy Sci. 69:1793–1798.
- Mayer, H. K., M. Ortner, E. Tschager, and W. Ginzinger. 1997. Composite milk protein phenotypes in relation to composition and cheesemaking properties of milk. Int. Dairy J. 7:305–310.
- Neumaier, A., and E. Groeneveld. 1998. Restricted maximum likelihood estimation of covariances in sparse linear models. Genet. Sel. Evol. 30:3–26.
- Ng-Kwai-Hang, K. F., J. F. Hayes, J. E. Moxley, and H. G. Monardes. 1986. Relationships between milk protein polymorphisms and major milk constituents in Holstein-Friesian cows. J. Dairy Sci. 69:22–26.
- Ojala, M., T. R. Famula, and J. F. Medrano. 1997. Effects of milk protein genotypes on the variation for milk production traits of Holstein and Jersey cows in California. J. Dairy Sci. 80:1776– 1785.
- Okigbo, L. M., G. H. Richardson, R. J. Brown, and C. A. Ernstrom. 1985. Casein composition of cow's milk of different chymosin coagulation properties. J. Dairy Sci. 68:1887–1892.
- Oloffs, K., H. Schulte-Coerne, K. Pabst, and H. O. Gravert. 1992. Die Bedeutung der Proteinvarianten f
  ür genetische Unterschiede in der K
  äsereitauglichkeit der Milch. Zuchtungskunde 64:20–26.
- Schmit, T. M., and H. M. Kaiser. 2006. Forecasting fluid milk and cheese demands for the next decade. J. Dairy Sci. 89:4924–4936.
- Threadgill, D. W., and J. E. Womack. 1990. Genomic analysis of the major bovine milk protein genes. Nucleic Acids Res. 18:6935– 6942.
- Wedholm, A., L. B. Larsen, H. Lindmark-Månsson, A. H. Karlsson, and A. Andrén. 2006. Effect of protein composition on the cheesemaking properties of milk from individual dairy cows. J. Dairy Sci. 89:3296–3305.