Associations Between Milk Protein Genotypes And Milk Coagulation Properties Of Estonian Holstein Cows

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Introduction

Different protein structures are appropriate for technological use in cheesemaking (Ikonen *et al.* (1999); Comin *et al.* (2008)) and nutrition (EFSA (2004)) due to genetic variation in milk proteins. The four casein genes (*CSN1S1*, *CSN2*, *CSN1S2*, and *CSN3*) are tightly linked in a cluster (Ferretti *et al.* (1990)) mapped on chromosome 6 and encode α_{s1}-CN, β-CN, α_{s2}-CN, and κ-CN, respectively (Hayes *et al.* (1993)). The two main whey proteins, α-LA and β-LG, are coded by *LAA* and *LGB* genes, mapped on chromosomes 5 (Hayes *et al.* (1993)) and 11 (Hayes and Petit (1993)), respectively.

The objective of this study was to investigate which aggregate β-κ-CN and β-LG genotypes or individual alleles are favourably associated with the milk coagulation and quality traits of Estonian Holstein cows.

Material and methods

Sampling and DNA analysis. Blood samples (n=2959) were collected as part of a development project for the Bio-Competence Centre of Healthy Dairy Products in Estonia during the period June 2005 to December 2007. Polymorphisms of three milk protein genes were analyzed, two from casein cluster (β-CN, κ-CN) and β-LG. DNA was extracted from whole blood according to Miller *et al.* (1988) or by using a commercial Puregene Gentra Blood kit (Minneapolis USA). On the basis of five SNPs in the casein cluster and one in β-LG gene, PCR-RFLP and ASO-PCR were carried out to detect the gene variants. Data from Chessa *et al.* (2007) and Ibeagha-Awemu *et al.* (2007) were considered when selecting the polymorphic sites to distinguish the protein variants.

Data collection and milk analysis. First lactation milk samples were collected during routine milk recording as part of a development project for the Bio-Competence Centre of Healthy Dairy Products in Estonia during the period April 2005 to June 2008. Milk samples with pH lower than 6.5 and non-coagulated milk samples (n=33) were excluded. Further, farms with less than 10 cows and cows with fewer than 3 test-day records were removed. The final dataset used for analyses consisted of 11,437 test-day records from 2,769 Estonian

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Holstein cows which were located in 66 herds across the country and were the daughters of 230 sires. The number of daughters per sire ranged from 1 to 169. Each cow had 3-6 measurements collected during different stages (7-305 days in milk) of the first lactation. Information about the cows, herds and pedigree was obtained from the Estonian Animal Recording Centre (EARC) and from the Animal Breeders' Association of Estonia. The test-day milk yield was recorded and individual milk samples were analyzed for protein percentage using the MilkoScan 4000 and MilcoScan FT6000, and for SCC using the Fossomatic 4000 and Fossomatic 5000 cell counters at the Milk Analysis Laboratory of EARC. Values of SCC were log-transformed to SCS as: $SCS = \log_2(SCC/100{,}000) + 3$. Milk coagulation properties (curd firmness in volts and coagulation time in minutes) were determined at the Laboratory of Milk Quality of the Estonian University of Life Sciences on an average 3.5 (SD=1.6) days after sampling. The proportion of milk samples with an age over seven days was less than 1%. Prior to the assessment of the milk coagulation properties (MCP), the milk samples were heated to the renneting temperature (35 ˚C). The rennet (Milase MRS 750 IMCU/ml; CSK Food Enrichment B.V., The Netherlands) used in the analyses was diluted 1:100 (v/v) with distilled water and 0.2 ml of the solution was added to 10 ml milk. MCP were determined using the Optigraph (Ysebaert, Frepillon, France), developed by YDD (Ysebaert Dairy Division) in partnership with the INRA (LGMPA, lab. G. CORRIEU).

Statistical analyses. Preliminary analyses for testing significance of fixed effects and single genotype effects were carried out in SAS System (SAS Institute, Inc., Cary, NC, USA) using MIXED procedure. Aggregate β-κ-CN genotypes were formed for further analysis. The genotypes with relative frequency less than 1% were grouped together to rare β-κ-CN genotype (A^1A^1/BB, A^1A^1/BE, A^1A^1/EE, A^1B/BB, A^1B/BE, A^2A^2/AE, A^2A^2/BE, A^2A^3/AA, A^2B/AA, A^2B/BB, BB/AB, BB/BB). Further statistical analysis was carried out in ASReml (VSN International Ltd., Hemel Hempstead, UK), using the following univariate repeatability animal model:

$$\mathbf{y = Xb + Cg + Za + e,}$$

where **y** – vector of observations of dependent variable (log-transformed RCT, A_{30}, milk yield, milk protein percentage); **b** – vector of fixed effects (quadratic polynomial of days in milk, calving age, sample age, sampling year-season, calving year-season); **g** – fixed effects of the β-κ-CN genotypes or β-LG genotypes; **a** – vector of random effects (herd $N(0,\mathbf{I}\sigma_h^2)$, additive genetic $N(0,\mathbf{A}\sigma_a^2)$ and permanent environmental effect $N(0,\mathbf{I}\sigma_{pe}^2)$); **e** – vector of residual random error effects $N(0,\mathbf{I}\sigma_e^2)$; **X**, **C**, **Z** – known incidence matrixes for fixed, genotype and random effects, respectively.

Sample age was included as a covariate in the model only for milk coagulation traits. Sampling year-season and calving year-season were grouped into 3-month classes, 14 classes from April 2005 to June 2008 and 11 classes from December 2004 to August 2007, respectively. Three generations of ancestors with total number of 17,185 animals in the relationship matrix were included in the analysis.

Results and discussion

The most frequent κ-CN genotype of all genotyped Estonian Holstein cows was AA, which was found in slightly more than half of the cows. The most common β-CN genotypes A^1A^2 and A^2A^2 had nearly equal frequencies and comprised jointly 84%. The rare EE genotype of

κ-CN occurred only in combination with genotype A^1A^1 of β-CN, while the rare A^2A^3 genotype of β-CN combined only with AA genotype of κ-CN. Preliminary analysis revealed association for SCS only with κ-CN genotype (p=0.02). Further analyses indicated significant effect of β-κ-CN genotypes on milk coagulation traits (p<0.001), milk yield (p=0.015) and protein percentage (p<0.001) as presented in Table 1.

Table 1: The number of Estonian Holstein cows (n) per β-κ-CN aggregate genotype and β-LG genotype and estimated genotype effects (±SE) on milk coagulation time (RCT), curd firmness (A_{30}) and milk production and protein percentage in milk sample data

Genotype	n	RCT* (min)	A_{30} (V)	Milk (kg)	Protein (%)
β-/κ-CN					
A^1A^1 /AA	110	- 0.04±0.01	- 0.51±0.25	- 1.24±0.42	0.01±0.02
A^1A^1 /AB	42	- 0.04±0.02	2.16±0.39	- 0.70±0.65	0.07±0.03
A^1A^1 /AE	70	- 0.03±0.02	- 0.89±0.31	- 1.13±0.51	0.05±0.02
A^1A^2 /AA	633	- 0.02±0.01	- 0.24±0.13	- 0.64±0.22	0.02±0.01
A^1A^2 /AB	271	- 0.06±0.01	2.40±0.17	- 0.71±0.29	0.05±0.01
A^1A^2 /AE	207	- 0.01±0.01	- 0.68±0.19	0.03±0.32	- 0.00±0.01
A^1A^2 / BB	34	- 0.07±0.02	4.36±0.42	- 2.01±0.70	0.13±0.03
A^1A^2 / BE	49	- 0.05±0.02	2.21±0.36	- 0.96±0.59	0.04±0.03
A^1B /AB	42	- 0.14±0.02	2.39±0.39	0.06±0.65	0.03±0.03
A^2A^2 /AA	768	0	0	0	0
A^2A^2 /AB	337	- 0.02±0.01	2.16±0.16	- 0.38±0.27	0.06±0.01
A^2A^2 / BB	34	- 0.07±0.02	3.87±0.42	- 0.46±0.70	0.08±0.03
A^2B /AB	75	- 0.09±0.02	1.97±0.30	- 0.44±0.49	0.01±0.02
Rare**	93	- 0.08±0.01	2.02±0.27	- 0.45±0.45	0.03±0.02
β-LG					
AA	589	- 0.02±0.01	- 0.43±0.13	0.18±0.19	- 0.00±0.01
AB	1569	0	0	0	0
BB	609	0.03±0.01	0.37±0.13	0.20±0.20	- 0.01±0.01

* Log-transformed
** Genotypes with occurrence less than 1%

The most favourable β-κ-CN genotypes for RCT included B allele in both loci as reported also by Comin *et al*. (2008) for Italian Holstein. Superior aggregate genotypes for A_{30} had at least one B allele of κ-CN. The most frequent β-κ-CN genotype, A^2A^2/AA, and genotype A^1A^2/AE were associated with poor milk coagulation properties, which is consistent with Comin *et al*. (2008). Also rare E allele of κ-CN in β-κ-CN genotype had rather unfavourable effect on milk coagulation properties. Association of this rare allele of κ-CN with poor milk coagulation is previously reported in studies by Ikonen *et al*. (1999) and Comin *et al*. (2008). The most frequent β-κ-CN genotype, A^2A^2 /AA, is favourable for milk yield. Similarly to A_{30}, the most favourable β-κ-CN genotypes for milk protein percentage were homozygous for B allele of κ-CN. Genotypes of β-LG were associated with milk coagulation traits (p<0.001), but did not have a significant effect on milk yield (p=0.462) and protein

percentage (p=0.648). Firmer curd was associated with BB genotype of β-LG, whereas somewhat shorter milk coagulation time was associated with AA genotype of β-LG.

Conclusion

Preliminary analysis revealed association for SCS only with κ-CN genotype. β-κ-CN genotype affected milk coagulation traits, milk yield and protein percentage, whereas β-LG genotype was associated only with milk coagulation traits. The most frequent β-κ-CN genotype, A^2A^2/AA, was associated with rather poor milk coagulation properties and with higher milk yield.

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