# Expression polymorphism of κ-casein gene in Holstein cows

By GILLES ROBITAILLE and DENIS PETITCLERC

*Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre, PO Box 90, Lennoxville, Canada J1M 1Z3*

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Several studies have reported associations between κ-casein (κ-CN) genetic variants A and B and milk composition and properties (for review, see Ng-Kwai-Hang, 1998). For instance, genetic variant κ-CN B was associated with higher protein, casein and κ-CN contents. The objective of the present study was to demonstrate a differential expression of κ-CN genetic variants A and B at the mRNA level in mammary epithelial cells during lactation.

## MATERIALS AND METHODS

Eighteen lactating Holstein cows, heterozygous AB for κ-CN and belonging to the herd of the Dairy and Swine Research and Development Centre, were included in the study. They were unrelated up to the third generation except that two grandsires were genitors for more than one cow. Biopsies were performed on mammary glands using a Tru-cut™ biopsy needle (VWR, Mississauga, Canada L5N 5Z7) and total RNA was extracted from the tissue using Trizol™ reagent (Gibco-BRL, Burlington, Canada L7P 1A1).

A combination of reverse transcriptase polymerase chain reaction (RT-PCR) and single-strand conformational polymorphism gel analysis (SSCP) was used to quantify allele-specific mRNA (Maekawa *et al*. 1996). Primers in exons IV and V ($^{10551}$TGTGCTGAGTAGGTATCCTAGTTATGG$^{10577}$ and $^{12929}$GTTTGAAGTAGTC-ATTTGGTTTCAGC$^{12954}$ respectively; Alexander *et al*. 1988; GenBank accession no. X14908) were selected to amplify the regions within the κ-CN gene where mutations giving genetic variants are located. Primers were end-labelled (Ready-to-Go T4 polynucleotide kinase labelling kit; Pharmacia Biotech, Pointe-Claire, Canada H3H 4H4) using [$\gamma$-$^{32}$P]ATP (specific activity 6000 Ci/mmol; Amersham Canada, Oakville, Canada L6L 5T7). RT-PCR was carried out on 1 $\mu$l total RNA in 25 $\mu$l reaction mix (Titan® one tube RT-PCR kit; Boeringher Mannheim, Laval, Canada H7V 4A2) in a PTC 200 thermocycler (MJ Research Inc., Watertown, MA 02172, USA). After incubation at 50 °C for 30 min, PCR was carried out as follows: 10 cycles of amplification (30 s at 94 °C, 30 s at 55 °C and 45 s at 68 °C), 15 cycles of amplification with the same denaturation and annealing conditions but with an elongation time increasing by 5 s at each cycle, and a final elongation of 7 min at 68 °C. SSCP analysis was carried out at 15 °C as described by Barroso *et al*. (1997), modified to include a stacking gel containing 0·5 l/l formamide (Yap & McGee, 1993). The gel was exposed to Biomax film MS (Eastman Kodak Co., New Haven, CT 06511, USA) for $\sim$ 24 h. The autoradiographic images were scanned and analysed using a computerized scanning densitometer and software (GS-670 densitometer;

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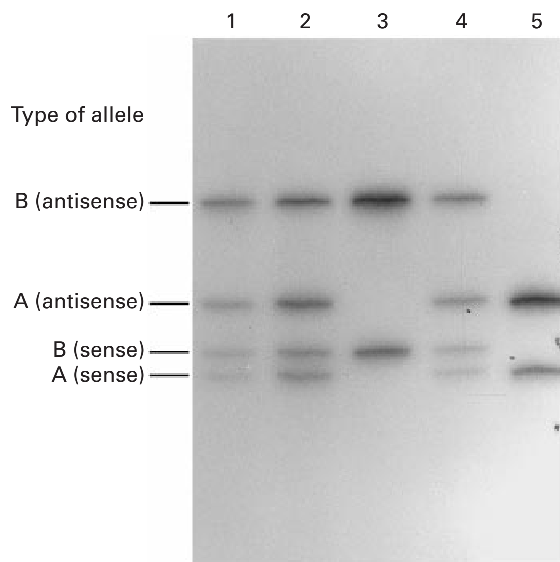


Fig. 1. Single-strand conformational polymorphism analysis of $\kappa$-casein transcripts amplified by reverse-transcriptase polymerase chain reaction of total RNA extracted from mammary gland biopsies. Cows were genotyped AB (lanes 1, 2 and 4), BB (lane 3) and AA (lane 5) for $\kappa$-casein.

BioRad, Richmond, CA 94804, USA) to integrate the bands corresponding to single stranded DNA (ssDNA) from alleles A- and B-specific mRNA. The relative percentage of ssDNA B to total ssDNA (ssDNA A + ssDNA B) was calculated; this corresponded to the relative percentage of $\kappa$-CN allele B-specific mRNA in the $\kappa$-CN mRNA population.

Milk samples were collected from 13 cows before biopsy. Casein fractions were obtained from skim milk by isoelectric precipitation, dissolved at 25 mg/ml in 0·01 M-sodium phosphate–0·15 M-NaCl, pH 6·5 and treated at 37 °C for 1 h with 0·05 i.u. *Clostridium perfringens* neuraminidase (Boehringer Mannheim) and *Arthrobacter ureaplasma* neuraminidase (Sigma Chemical Co., St Louis, MO 63178, USA) to remove sialic acid moieties on $\kappa$-CN. Milk caseins were fractionated by isoelectric focusing PAGE as described by Seibert *et al.* (1985) using a Multiphor II electrophoresis system (Pharmacia Biotech). The gels were stained with Coomassie blue and scanned. The bands corresponding to $\kappa$-CN protein variants A and B were integrated to obtain their relative amounts of total $\kappa$-CN.

The classification of the 18 cows into two groups based on the relative amounts of $\kappa$-CN B mRNA to total $\kappa$-CN mRNA was evaluated by a cluster analysis and the relative percentage of $\kappa$-CN protein variant B to whole $\kappa$-CN in milk for each group of cows was compared using a $t$ test (SAS, 1997).

## RESULTS AND DISCUSSION

Typical autoradiographic patterns of RT-PCR–SSCP analysis are shown in Fig. 1 and the relative percentages of allele B-specific mRNA are given in Table 1 (means of three distinct RT-PCR–SSCP). The distribution of the values suggested bimodality, confirmed using the cluster analysis. For ten cows (group 1), the amounts of $\kappa$-CN allele A- and B-specific mRNA were similar. For the remaining eight cows (group 2), there was more mRNA transcribed from allele B than from allele A. We

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Table 1. *Relative amounts of κ-casein allele B-specific mRNA to total κ-casein mRNA extracted from mammary tissue and of κ-casein B genetic variant in the milk of heterozygous κ-casein AB cows*

| Group 1† | | | | | Group 2† | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cow no. | Lactation no. | Days in milk | B-specific transcripts, % of total | κ-Casein B genetic variant, % of total | Cow no. | Lactation no. | Days in milk | B-specific transcripts, % of total | κ-Casein B genetic variant, % of total |
| 301 | 3 | 175 | 48·7 | | 326 | 2 | 278 | 58·3 | |
| 458 | 1 | 192 | 50·7 | 54·9 | 437 | 1 | 167 | 57·7 | |
| 463 | 1 | 146 | 51·6 | 59·3 | 452 | 1 | 177 | 56·7 | 59·3 |
| 5007 | 3 | 297 | 50·6 | 59·5 | | 2‡ | 70 | 56·2 | |
| 5008 | 3 | 246 | 50·2 | 55·6 | 461 | 1 | 137 | 54·9 | 56·1 |
| | 4 | 297 | 49.9 | | | 2 | 30 | 55·3 | |
| 5017 | 2 | 242 | 50·8 | | 5013 | 4 | 262 | 55·5 | 60·4 |
| 5060 | 2 | 385 | 50·4 | 55·5 | 5033 | 2 | 204 | 57·6 | 60·4 |
| 5090 | 1 | 172 | 49·0 | 52·2 | | 3 | 172 | 56·7 | |
| | 2 | 21 | 49·6 | | 5068 | 2 | 257 | 55·1 | 60·0 |
| 5103 | 3 | 125 | 48·9 | 57·9 | 5102 | 3 | 137 | 56·9 | 65·0 |
| 5100 | 2 | 153 | 50·4 | | | | | | |
| Mean ± SD | | | 50·1 ± 0.9 | 56·4 ± 2·6 | | | | 56·6 ± 1·3 | 60·2 ± 2·9 |

† Groups were defined on the basis of the percentage of κ-casein allele B-specific mRNA.
‡ Values obtained from cows analysed in subsequent lactations are not included in the calculation of the means.

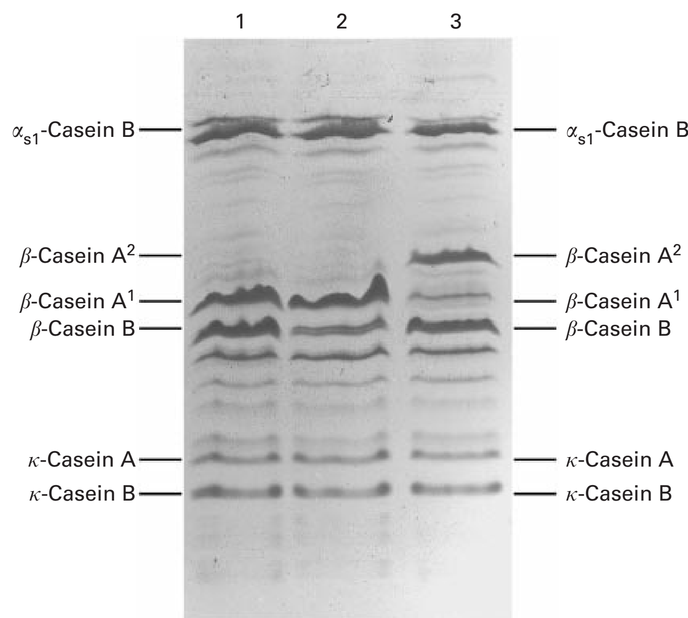


Fig. 2. Isoelectric focusing polyacrylamide gel electrophoresis of caseins. The location of protein variants for $\alpha_{s1}$-, $\beta$- and $\kappa$-casein is given. Cows were genotyped BB and AB for $\alpha_{s1}$- and $\kappa$-casein respectively and $A^1B$ (lane 1), $A^1A^1$ (lane 2) and $A^2B$ (lane 3) for $\beta$-casein.

did not observe any effects of either lactation stage or parity on the allocation of the cows between the two groups. For instance, the relative percentage of $\kappa$-CN allele B-specific mRNA was not modified for five cows that were analysed during a subsequent lactation (Table 1).

We also determined the relative percentages of $\kappa$-CN A and B genetic variants in the milk of 13 heterozygous cows (group 1, $n = 7$; group 2, $n = 6$) taken just before biopsy (Table 1); a typical isoelectric focusing PAGE is shown in Fig. 2. The tendency toward a higher $\kappa$-CN B genetic variant content in the milk of heterozygous cows, previously reported by Van Eenennaam & Medrano (1991), was confirmed in this study. The overall mean ($\pm$SD) for the relative percentage of $\kappa$-CN B genetic variant was $58{\cdot}1 \pm 3{\cdot}3\%$. Interestingly, the relative percentages of the $\kappa$-CN B genetic variant in the milk from cows of groups 1 and 2 were significantly different ($P < 0{\cdot}05$; Table 1).

In conclusion, although all the cows in this study were genotyped $\kappa$-CN AB, the pattern of expression of the alleles coding for $\kappa$-CN could differ. For some cows (group 2) the allele B-specific gene was overexpressed into mRNA compared with the allele A-specific gene. The cows from group 1, however, had similar levels of expression for both alleles. To explain the difference in the expression of allele A- and B-specific $\kappa$-CN genes in the mammary gland of heterozygous cows, we propose that one allele was polymorphic in the non-coding region of the gene. This polymorphism would affect the transcriptional activity of the gene, the splicing process or the mRNA stability. Hence, for heterozygous $\kappa$-CN AB cows, the contents of allele A- and allele B-specific mRNA would be either similar when the two alleles had the same DNA sequence within the polymorphic region (group 1) or different when one of the two alleles carried the mutation that caused the differential allelic expression of the $\kappa$-CN gene. It is notable that the polymorphism causing the differential allelic expression

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of κ-CN gene was not linked to the polymorphism responsible for the presence of genetic variants of κ-CN A and B in milk. We are currently sequencing the κ-CN gene of heterozygous cows to search for this expression polymorphism.

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