# Molecular genetic polymorphism at the κ-casein and β-lactoglobulin loci in Finnish dairy bulls

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Dairy bulls have been genotyped for κ-casein and β-lactoglobulin from semen samples by methodology based on a polymerase chain reaction (PCR). In this study, a previously described method for κ-casein A and B variants was extended to cover also the detection of the E variant. For β-lactoglobulin the variants A and B were genotyped by another PCR-based method. The frequencies of the κ-casein and β-lactoglobulin alleles were determined from 308 and 291 Finnish Ayrshire and 42 and 44 Finnish Friesian bulls, respectively. The bulls had been born between 1973 and 1988. There was no noticeable trend in the differences between allele frequencies over the years, the overall frequencies of κ-casein A, B and E being 0.62, 0.09 and 0.29 in the Finnish Ayrshires and 0.85, 0.14 and 0.01 in Finnish Friesians. The overall frequencies of β-lactoglobulin A and B alleles were 0.25 and 0.75 in Ayrshires and 0.56 and 0.44 in Friesians.

Key words: dairy cattle, PCR, κ-casein, β-lactoglobulin, genetic variation

## Introduction

The genetic polymorphism of the four major casein proteins ($\alpha_{S1}$, $\alpha_{S2}$, β and κ) and two whey proteins (α-lactalbumin and β-lactoglobulin) in cattle is well documented (GROSCLAUDE 1988). It derives both from substitutions and deletions in the amino acid sequences of these proteins (EIGEL et al. 1984). Protein electrophoresis has been used to identify the different protein variants of bovine milk. This kind of methodology for the determination of bull genotypes requires the analysis of multiple dam/daughter pairs, thereby considerably delaying the results. To overcome these restrictions, DNA analysis techniques have been applied to type animals for milk protein loci (LEVÉZIEL et al. 1988, ROGNE et al. 1989). Among DNA amplification techniques, several PCR-based methods for genotyping milk protein loci have been published (MEDRANO and AGUILAR-CORDOVA 1990a, 1990b, DAVID and DEUTCH 1992, LIEN et al. 1992).

We have recently initiated a study to analyze the associations between production traits and milk protein genotypes in Finnish dairy cattle. The present paper is the first phase of the work. Here we describe some modifications to existing PCR-based methodology and present the allele frequencies of κ-casein (CASK) and β-lactoglobulin (LGB) for the major Finnish dairy breeds from a sample of artificial insemination (AI) bulls.

## Material and methods

Frozen semen pellets from altogether 324 Finnish Ayrshire and 53 Finnish Friesian bulls were ob-

tained from five Finnish AI societies. The birth years of the bulls ranged from 1973 to 1988. Ayrshire bulls were divided into three age groups according to their birth year: 1973-81, 1982-85 and 1986-88.

The method described by ZADWORNY and KUHNLEIN (1990) was applied to isolate DNA from the semen. The DNA concentration was determined by fluorescence (Hoefer TKO 100 Fluorometer). The yield was in the range of 10-30 µg DNA per semen pellet. The isolation of DNA from whole blood was done with a rapid method described by KAWASAKI (1990).

The PCR reactions, restriction enzyme digestions and gel runs for CASK A and B variants and for LGB A and B variants were performed under the conditions described by MEDRANO and AGUILAR-CORDOVA (1990a, 1990b), with following modifications: The amount of DNA in the reaction was 100 ng; the reaction volume was 25 µl; the reaction buffer did not contain gelatin; and 1.25 units of *Taq* DNA polymerase (Promega) were used per reaction. The oligonucleotides used for the amplification of CASK were

K346A (5'-CATTTATGGCCATTCCACCAAAG-3')
and
K346B (5'-CATTTCGCCTTCTCTGTAACAG-3').

These primers are otherwise the same as JK501 and JK302 (MEDRANO and AGUILAR-CORDOVA 1990a) but they are 2 and 4 bases shorter. Also, K346B is an inversion of the JK302 orientated erroneously, as could be deduced from the κ-casein sequence (ALEXANDER et al. 1988). For CASK the PCR programme consisted of denaturation for 3 min at 94°C followed by 1 min at 94°C, 50 sec at 60°C, 30 sec at 72°C for 10 cycles, 1 min at 94°C, 50 sec at 60°C, 45 sec at 72°C for 20 cycles, 1 min at 94°C, 50 sec at 60°C, 1 min at 72°C for 10 cycles. A final extension of primers for 5 min at 72°C was included for both PCR programmes. To improve specificity and yield of the reaction, the 'hot start' technique was applied to start the reactions: the nucleotides needed for amplification were added during the first denaturation step of the PCR programme. The amplifications were performed with a Hybaid Thermal Reactor (Hybaid Limited, Middlesex, UK).

CASK E variant has been genotyped at DNA level using the restriction enzyme *Hae* III (SCHLIEBEN et al. 1991). We found that with the PCR-product obtained by the method of MEDRANO and AGUILAR-CORDOVA (1990a), also a specific banding pattern for E variant could be observed after digestion with *Hae*III. Thus, the method for CASK was extended to cover also the detection of the E variant. An aliquot of 10 µl of the PCR product was used for all restriction enzyme digestions.

Prior to this study, 20 cows of Finnish Ayrshire breed with known CASK and LGB genotypes had been tested with the above genotyping assays from blood samples. The results deduced from the DNA analysis were identical to those obtained at protein level.

## Results and discussion

In addition to CASK A and B variants we also detected the E variant. The mutation in codon 155 (Ser to Gly) is known to be responsible for this variant (ERHARDT 1989). The digestion of the CASK PCRproduct (346 bp) with *Hae*III resulted in fragments 337 bp (and 9 bp) for non-E and 192 bp, 145 bp (and 9 bp) for the E variant. Bands were easily distinguishable since a strong amplification product was obtained in most cases (Figure 1). The polymorphism at codon 148 was detected using the procedure of MEDRANO and AGUILAR-CORDOVA (1990a) for CASK A and B variants. The polymorphism at codon 118 was utilized for LGB (MEDRANO and AGUILAR-GORDOVA 1990b).

A successful amplification was obtained from 308 and 291 Ayrshire samples and from 42 and 44 Friesian samples in CASK and LGB, respectively. The allele distributions calculated from these results are presented in Table 1. For the Finnish Ayrshire breed, the allelic frequencies of A, B and E at the CASK locus were 0.61, 0.10 and 0.29, and the frequencies of A and B at the LGB locus were 0.25 and 0.75. For the Finnish Friesian, the allelic frequencies of A, B and E at CASK were 0.85, 0.14

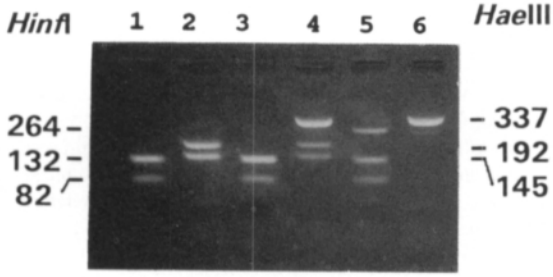


Fig. 1. Discrimination of κ-casein genotypes as detected by agarose gel electrophoresis of *Hinf*I (lanes 1, 3, 5) and *Hae*III digested (lanes 2, 4, 6) PCR products. The three samples presented here were typed to be DNA genotypes EE (lanes 1 and 2), AE (lanes 3 and 4) and AB (lanes 5 and 6) of the CASK gene. (Photo: R. Velmala).

and 0.01 and those of A and B at LGB were 0.56 and 0.44.

Our results for the overall allele frequencies of CASK for Ayrshires and Friesians are in agreement with previous results obtained for these breeds by protein electrophoresis (PIIRONEN et al. 1992). In Ayrshires, there was some variation in the frequency of A and E allele between the age groups. These differences, however, did not represent any trend in time. There had been no drastic alterations in selection criteria over the studied period, so that the changes could not be explained as being resulting from a correlated response to selection for production. The frequency fluctuations are most likely due to genetic sampling involving only a small number of parents sireing the bulls analyzed.

In LGB, there were no noticeable differences in allele frequencies between the age groups. The frequency of LGB B allele, however, was somewhat higher in Ayrshires and lower in Friesians compared to previous results for these breeds (AALTONEN and ANTILA 1987, ATROSHI et al. 1982, PIIRONEN et al. 1992, TERVALA et al. 1983). The differences here, too, may be due to genetic sampling. On the other hand, the present study was made with bulls whereas the previous ones have dealt with cows. Another factor is that in the 1980's there were substantial semen contributions by Holstein bulls into the Friesian population, which may have changed the allele distribution in Friesian bulls.

Several studies have investigated the association between milk protein variants and various economically important milk traits, including manufacturing properties (e.g. AALTONEN and ANTILA 1987, NG-KWAI-HANG et al. 1990). Current molecular genetic techniques allow a thorough screening of genetic variation among AI-bulls for milk protein loci. The analyses presented here clearly indicate that a routine and inexpensive testing for CASK and LGB is feasible. However, a more direct and detailed analysis of the association between the economic traits and milk protein variants is required. In that context, the CASK locus should be analyzed together with other closely linked casein loci (LIEN et al. 1993).

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Table 1. Number of samples and allele frequencies of κ-casein and ß-lactoglobulin loci in AI bulls by breed and age group.

| | | κ-casein | | | β-lactoglobulin | | |
|---|---|---|---|---|---|---|---|
| | | A | B | E | | A | B |
| Finnish Ayrshire | No. | | | | No. | | |
| 1973-81 | 82 | 0.71 | 0.07 | 0.23 | 75 | 0.26 | 0.74 |
| 1982-85 | 85 | 0.52 | 0.11 | 0.36 | 81 | 0.28 | 0.72 |
| 1986-88 | 141 | 0.62 | 0.10 | 0.27 | 135 | 0.22 | 0.78 |
| total | 308 | 0.62 | 0.10 | 0.29 | 291 | 0.25 | 0.75 |
| Finnish Friesian | | | | | | | |
| 1975-88 | 42 | 0.85 | 0.14 | 0.01 | 44 | 0.56 | 0.44 |

No.=number of animals analyzed.

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SELOSTUS

# κ-kaseiinin ja β-laktoglobuliinin varianttien molekyyligeneettinen tunnistaminen ja muuntelu suomalaisissa lypsykarjasonneissa

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Maatalouden tutkimuskeskus

Kotieläintuotannon tutkimuslaitoksen eläinjalostusyksikössä on aloitettu tutkimus maitoproteiinivarianttien yhteyksistä eläinten tuotanto-ominaisuuksiin. Tässä julkaisussa esitetään työn ensimmäinen vaihe, jossa molekyyligeneettistä menetelmää sovellettiin keinosiemennyssonnien κ-kaseiinin ja β-laktoglobuliinin genotyypitykseen. Tuloksista laskettiin näiden lokusten alleelifrekvenssit ayrshire- ja friisiläisroduille.

DNA-monimuotoisuutta analysoitiin entsymaattisen monistamisen (PCR) ja DNA:ta spesifisesti pilkkovien entsyymien avulla. κ-kaseiinin variantit A, B ja E sekä β-laktoglobuliinin variantit A ja B määritettiin spermanäytteistä. Yhteensä 308/291 ayrshire- ja 42/44 friisiläisnäytettä analysoitiin κ-kaseiinin/β-laktoglobuliinin suhteen. κ-lokuksen A-, B- ja E-alleelien frekvenssit olivat ayrshire-sonneilla 0,61, 0,10 ja 0,29 ja friisiläissonneilla 0,85, 0,14 ja 0,01. β-laktoglobuliinilokuksessa olivat A- ja B-alleelien frekvenssit ayrshirella 0,25 ja 0,75 ja friisiläisillä vastaavasti 0,56 ja 0,44. Alleelifrekvenssejä tutkittiin ayrshirellä myös ikäryhmittäin sonnien syntymävuosien (1973-88) mukaan. Frekvensseissä ei havaittu systemaattista muutosta tutkittuna ajanjaksona. Näiden rotujen κ-lokuksen alleelifrekvenssit vastasivat hyvin jo aiemmin maidosta proteiinielektroforeesilla määritettyjä frekvenssejä. β-laktoglobuliinin alleelifrekvensseissä havaittiin vähäisiä eroja aikaisempiin tutkimuksiin verrattuna. Erot saattavat johtua analysoidun aineiston koosta. Lisäksi aikaisemmat tutkimukset on tehty lehmillä kun taas tässä tutkimuksessa käytettiin sonneja.

Maitoproteiinialleelien ja taloudellisten ominaisuuksien välisistä yhteyksistä tarvitaan yksityiskohtaisempaa analyysiä. Tällaisessa analyysissä κ-kaseiinilokus pitäisi analysoida yhdessä muiden geneettisesti kytkeytyneiden kaseiinilokusten ($\alpha_{S1}$-, $\alpha_{S2}$- ja β-kaseiinin) kanssa.