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Dde I RFLP at the 5' region of bovine kappa-casein gene

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Abstract. The bovine kappa-casein (CASK) gene is known as a potential quantitative trait locus in dairy cattle breeding. However, the molecular basis of the effect of the CASK allele B on different milk properties remains unclear. In this report, a 214 bp fragment of the 5' untranslated region of the CASK gene containing 5 potential consensus sequenses for different transcription factors was PCR-amplified to find RFLPs. A Dde I RFLP was identified. In a population of 112 Bos taurus (86 cows and 26 bulls of Polish Black and White crossbred Holstein-Friesian) and 7 Bison bonasus individuals, 7 had no recognition sites for Dde I, 23 were heterozygous and 89 were cut completely into two fragments.

Key words: bovine kappa-casein, Dde I, polymorphism, 5' untranslated region of gene.

Introduction

The CASK gene belongs to the cluster of four bovine casein genes located within a 200 kb fragment on chromosome 6 (FERETTI et al. 1990, THREADGILL, WOMACK 1990). The overall length of the CASK gene is close to 13 kb, but most of the sequence coding for the mature kappa-casein molecule is contained within the fourth exon (ALEXANDER et al. 1988). So far, four variants of the CASK protein (A, B, C, E) have been described (GROSCLAUDE et. al. 1972, ERHARDT 1989, CHIANESE et al. 1991).

Variant B of the CASK protein is favourable for milk composition (MAO et al. 1992), cheese-making properties and cheese yield (JAKOB, PUHAN 1992) and therefore CASK gene is considered a potential quantitative trait locus in dairy cattle (BOVENHUIS, WELLER 1994).

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However, the molecular nature of the effect of the CASK allele B on different milk properties remains unclear. The only hypothesis found in the literature suggests the association of the CASK allele B effect with potential mutations in the untranslated region of the gene which may affect the regulation of its expression (SCHILD et al. 1994).

In this paper a Dde I polymorphism within a specific 5' subregion of the CASK gene containing 5 potential consensus sequences of transcription factors is reported.

Material and methods

112 animals of Bos taurus (Polish Black and White crossbred Holstein-Friesian) and 7 individuals of Bison bonasus Białowieża were included in the analysis. Among Bos taurus, there were 26 A.I. bulls and 86 milking cows randomly chosen from three dairy herds located in Żuławy district. The Bison bonasus group consisted of 5 bulls and 2 cows from a free-living herd in Białowieża National Park.

DNA was isolated from commercial sperm portion or blood by the Easy Genomic DNA Prep Plus Kit and Blood DNA Kit (DNA-Gdańsk), respectively. Alternatively, leukocyte or sperm lysates were prepered as a DNA template for PCR mix (LIEN et al. 1990, HIGUCHI 1992).

Using Dnasis computer program the 214 bp DNA fragment located within the 5' region of the CASK gene, position 1617-1831 (GROENEN et al. 1991) was chosen and has been amplified by the PCR technique. PCR reaction was as follows: 2.5 µl 10x PCR, 1.5 µl dNTP-mix (2 mmol each), 0.5 µl of primer CASK-7 and 0.5 µl primer CASK-8 (100 ng/µl each), 0.5 u Taq polymerase (Promega), 1.0 µl of DNA template (100 ng/µl) or 0.5 µl lysate and H_20 to a volume of 25 µl. PCR program: predenaturation – 3 min 94°C followed by 35 cycles of: 30 sec 94°C, 30 sec 56°C, 30 sec 72°C. PCR primers: CASK-7: 5' GGA TCC CTA CTT TAT ATT GAT 3', CASK-8: 5' CAA TAG CAC TTT TAC ATT TCA 3'.

Seven µl of PCR products were digested with the Dde I restriction enzyme (Promega). The PCR products and restriction fragments were electrophorezed in 1.5% agarose/ethidium bromide gel. Gels were vizualisized and documentated by the GDS 7500 system (UVP).

Results and discussion

The effect of the CASK allele B is known to be economically important and it is considered to be included in diary cattle breeding programs (PEDERSEN

1991). However, in comparison to other casein genes, the regulation of expression of the kappa-casein gene is rather poorly investigated (GROENEN, POEL 1994). In the literature there is no explanation for the molecular nature of the effect of the CASK B allele on milk properties except the work of van EENENNAAM, MEDRANO (1991). These authors have assumed that the CASK B allele is more efficiently expressed than the A allele and the B allele is associated with a greater amount of total CASK protein present in the milk of AB cows. They refer the cause of differentiation of the CASK gene expression to potential differences in cis-acting sequences involved in a quantitative expression of the CASK gene. Similar assumption is mentioned in the work of SCHILD et al. (1994).

In the present report, the known 5' region of the CASK gene sequence has been screened by the Dnasis computer program (Hitachi) to find these 5' gene fragments which contained relatively many regulatory consensus sequences. Mutations found within these 5' flanking sequences might have a potential impact on different expression of the CASK gene. A subregion of 214 bp has been found to contain five consensus sequences, for Oct 1 (octamer binding site 1), PMF (pregnancy-specific mammary nuclear factor), PMF, PMF, AP-2 (activator protein 2) with the homology of 88%, 90%, 89%, 90%, 75%, respectively.

By the use of the PCR technique, 214 bp was amplified from the CASK gene, position 1617-1831 (GROENEN et al. 1991) and digested with several different restriction enzymes. Dde I endonuclease has been found to give restriction fragment length polymorphism within the 214 bp PCR product. Three different DNA band patterns in the electrophorgram were observed: 214 bp (–/– , fragment uncut), 164 bp + 50 bp (+/+ , fragment completely cut) and 214 bp + 164 bp + 50 bp (fragment partly digested, heterozygote) (Fig. 1). The identity of the amplified CASK gene fragment both from Bos taurus and Bison bonasus animals was confirmed by the digestion of 214 bp PCR product with different restriction enzymes and DNA sequencing (data not shown).

The genotypes of 112 Bos taurus, Polish Black and White crossbred Holstein-Friesian and 7 Bison bonasus Białowieża individuals were identified. The Bison bonasus animals were included in the analysis to obtain a highly divergent group. The frequency of the observed genotypes is shown in Table 1. The results show predominance of the +/+ Dde I genotype in the Bos taurus group. On the contrary, in the Bison bonasus group the +/+ Dde I genotype seems to be rare (1 out of 7). The small number of animals in the Bison bonasus group does not allow to compare differences in the frequency of Dde I genotypes between these two species. However, this kind of observations may be explained by natural genetic differences between Bos taurus and Bison bonasus.

A similar suggestion was made for wood bison and plains bison by CRONIN, COCKETT (1993) who found new restriction polymorphisms present only in the bison. The Dde I genotype is independent of the CASK exon IV genotype. It can be easily observed in the Bison bonasus group, in which all animals are BB in the CASK exon IV locus (data not shown), having different Dde I genotypes (Table 1). Because of the predominace of the CASK exon IV allele B in European bison reported also by SIPKO et al. (1994) and by BURZYNSKA, TOPCZEWSKI (1995), the Dde I RFLP polymorphism may be more suitable as a DNA marker utilized for monitoring homozygosity degree and for preserving Bison bonasus population.

Table 1. The number of animals with different Dde I genotype in 5' region of CASK gene

Group of animals	Dde I genotype		
	+/+	+/–	–/–
Bos taurus			
cows	64	17	5
bulls	24	24	–
Bison bonasus	1	4	2
Total	89	23	7

The localization of the Dde I polymorphism is in accordance with the study of SCHILD et al. (1994) who have detected DNA variation in the same site of the 5' region of CASK gene by the Sanger sequencing method. DNA polymorphisms in the 5' untranslated region have been detected in other milk genes, namely bovine beta-lactoglobulin (WAGNER et al. 1994), bovine alfa-laktoglobulin (BLECK, BREMEL 1993) and porcine alfa-lactoalbumin (BLECK et al. 1995). This direction of research on milk gene polymorphisms seems to be very desirable because it shows potential heterogeneity of milk genes which may be utilized in quantitative trait loci mapping.

Further studies have been undertaken to find another potential polymorphisms in the 214 bp subregion of 5' sequence of CASK gene and their relationships with cheese making properties in Holstein-Friesian cattle. These studies should clearify how important are the detected polymorphisms for the CASK gene expression and milk processing properties and whether they may be used as effective genetic markers in dairy breeding.

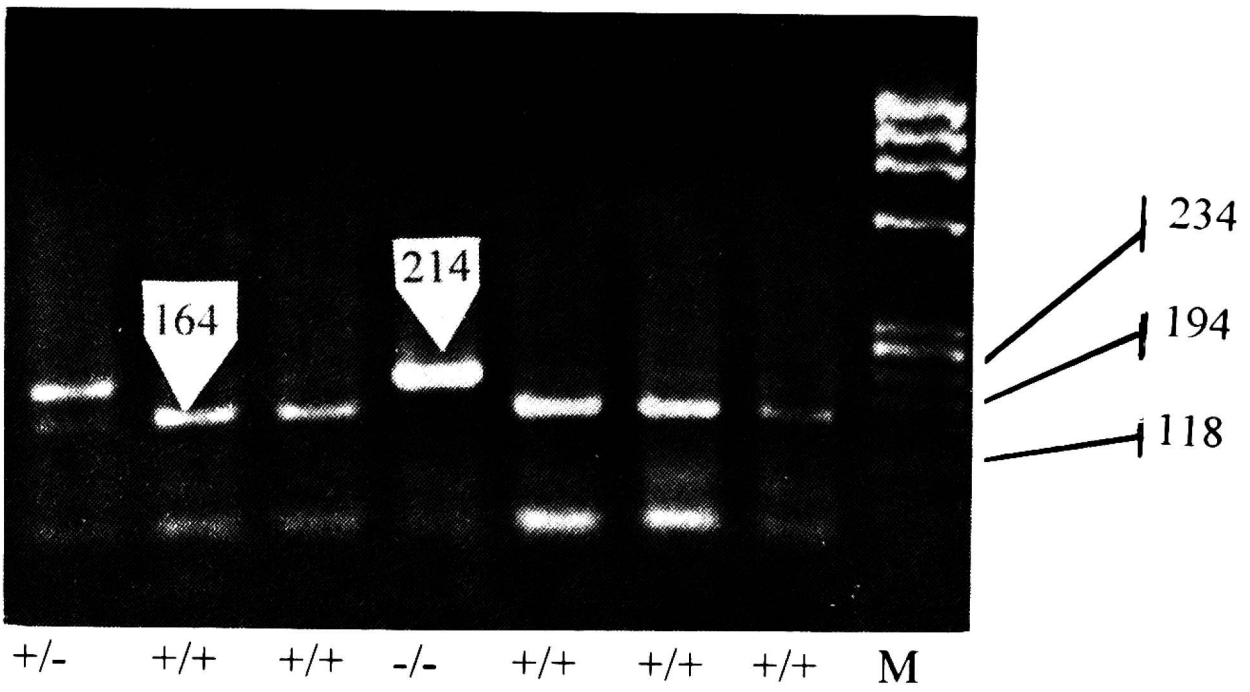

Fig. 1. Agarose gel containing 214 bp CASK gene fragment digested with Dde I. Restriction site presence (+) and absence (–) is identified for two alleles for eacl. ndividual sample. For example, +/– indicates an individual with one allele with Dde I site and one without the site. The numbers along the right side of the figure refer to the size of DNA fragments in base pairs and the lane labelled M contains a size marker PhiX 174 Hae III. The restriction fragment of 50 bp is not visible enough because of its small size and covering with slight primer-dimer complex.

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