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Original article

Association of nucleotide-sequence polymorphism in the 5'-flanking regions of bovine casein genes with casein content in cow's milk

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Abstract – The 5'-flanking regions (promoters) of the bovine α_{s1}-, α_{s2}- and β-casein genes were analysed for DNA sequence variants using PCR/RFLP in Polish Red (PR) and Black-and-White (BW) cattle. The polymorphic sites occurred at positions –1084 and –186 in the promoter region of the α_{s2}-casein gene and at –728 and –109 in the α_{s1}- and β-casein genes, respectively. These polymorphic sites were located within known potential regulatory sequences, suggesting an influence on the binding of transcription factors and expression of the bovine casein genes. Associations were found between various genotypes in the promoter region of the α_{s2}-casein gene and α_{s1}- and β-casein genotypes, thus showing the existence of intergenic haplotypes within the bovine casein locus. Milk proteins derived from cows of varying genotypes in the casein gene promoters were analysed using SDS-PAGE and HPLC techniques. It was shown that nucleotide sequence polymorphism in the promoter region of the bovine α_{s2}-casein gene was associated with various contents of α_{s2}- and β-casein in the milk.

Bovine casein gene / 5'-flanking region / gene polymorphism / milk protein

Résumé – Effet du polymorphisme de la séquence nucléotidique dans les régions 5'-non-codantes des gènes de la caséine bovine sur la teneur en caséine du lait de vache. Les variants de la séquence ADN dans les régions 5'-non codantes des gènes de caséines α_{s1}, α_{s2} et β des vaches de races pie-noir et rouge polonaises ont été analysés à l'aide de la méthode PCR/RFLP. Des polymorphismes ont été constatés dans les positions –1084 et –186 de la région promotrice du gène de la caséine α_{s2} et dans les positions –728 et –109 des gènes de la caséine α_{s1} et β, respectivement. Ces sites polymorphiques ont été localisés dans les séquences régulatrices potentielles, ce qui suggére leur influence sur la liaison des facteurs de transcription et l'expression des gènes de la caséine bovine. Des associations parmi les différents génotypes dans la région promotrice du gène de la caséine α_{s2} et les génotypes de la caséine α_{s1} et β ont été établies. Cela signifie que les haplotypes préférés existent dans le locus des caséines bovines. Les protéines du lait de vache de génotypes différents dans les promoteurs du gène de la caséine ont été analysées à l'aide de la technique SDS-PAGE et HPLC. Il y a été demontré que le polymorphisme de la séquence de nucléotides dans la région de promoteur du gène bovin de la caséine est lié avec les différentes teneurs en caséines α_{s2} et β dans le lait.

Gène de la caséine bovine / région 5'-non codante / polymorphisme / protéine du lait

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1. INTRODUCTION

In bovine milk, six major milk protein fractions – α_{s1}-, α_{s2}-, β-, κ-caseins, α-lactalbumin and β-lactoglobulin – exist in different allelic forms, which are controlled by codominant autosomal genes according to Mendelian inheritance. All four casein genes are clustered in a region less than 300 kb on bovine chromosome 6 in the order: α_{s1}, β, α_{s2} and κ [10, 26, 31]. It is assumed that the three genes coding for calcium-sensitive α_{s1}-, α_{s2}- and β-casein evolved from one ancestral gene by exon shuffling [16] and intra- and intergenic duplications [4, 33]. In contrast, the κ-casein encoding gene evolved differently [1]. Lien et al. [21] reported a strong genetic linkage of the casein genes by using a "single sperm typing" method. So, one may suppose that close localisation and linkage of the casein genes might influence a common inheritance of these genes. Moreover, the close proximity of the casein genes supported the hypothesis of common hormonal regulation of the entire complex [27].

Genetic polymorphism of milk proteins in cows, sheep and goats is well documented [13, 20, 24]. The proteins differ in amino acid substitutions or deletions, resulting from the differences in the coding sequences of corresponding genes. Associations have been described between polymorphism in the coding regions of the milk protein genes and their expression levels in cattle [32]. Variable sites have also been identified in the 5'-noncoding sequences of these milk protein genes [3, 6, 12, 17, 28, 29]. At least some of the genetically variable sites are located in putative regulatory sequences, e.g. in transcription factor-binding sites [28, 30], and thus they may influence gene expression. It has been suggested that differential expression of various milk protein alleles is a possible result of linkage between variants of coding and regulatory regions of their genes [32].

The objective of this study was to examine associations between polymorphisms in the 5'-flanking region of the bovine α_{s1}-, α_{s2}- and β-casein genes and the casein content in cow's milk.

2. MATERIALS AND METHODS

2.1. PCR-RFLP

Samples of blood were taken from Polish Red (PR) cows and heifers, maintained at the Research Station for Preserve Animal Breeding (Popielno); from animals kept by individual owners in the south of Poland (Podhale region); and from Polish Black-and-White (BW) cattle from the experimental station at Jastrzębiec. Numbers of animals used in individual experiments are given in tables and in the text. Genomic DNA was isolated from blood samples and collected on K_2EDTA as described previously by Kanai et al. [18]. The PCR-based detection of restriction fragment length polymorphism (RFLP) was carried out using primers and restriction endonucleases given in the paper by Schild and Geldermann [28]. The primer sequences and polymerase-chain-reaction (PCR) conditions are summarised in Table I. The PCR was performed in a reaction volume of 25 μL, containing ca. 100 ng of bovine genomic DNA, 0.25 μmol^{-1} of each primer, 160 μmol^{-1} dNTPs and 2 units of Taq polymerase. PCR-amplified α_{s2}-casein gene fragments –1150/+117 and –1150/–872, were digested at 37 °C for 3 h with 5 units of *Eco*RV and *Mae*II, respectively. For α_{s1}- and β-casein, respective gene fragments –1145/+101 or –1049/+87 were amplified and digested with *Ssp*I or *Nla*III endonucleases. The products of restriction digestion were separated on 2% agarose gels in 1× TRIS-borate-EDTA (TBE) buffer. All PCR reactions were performed using the MJ Research PTC-225 Thermal Cycler.

2.2. Analysis of milk proteins

Samples of milk were collected from PR and BW cows that were known from previous RFLP analyses to carry various genotypes in the α_{s2} and α_{s1} gene promoters. Protein separation was carried out using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and high performance liquid chromatography (HPLC).

Table I. PCR primers and conditions of DNA amplification.

Gene	Sequence of primers	Location	Amplified fragment (bp)	PCR cycle number	Time (min)		
					Temperature (°C)		
α_{s2}-casein	5'-TATGACATGTCGAGAAATGAG-3'	–1150/+117	1267		94	55	72
	5'-T TGGAACAATGCTATTAGGT T-3'			1	2.0	1.0	2.0
				2–34	1.0	1.0	1.0
β-casein	5'-GGTCTGGCTTAGACTCTATTG-3'	–1049/+87	1136	35	1.0	1.0	9.0
	5'-AGATGAGTAGCAGATACAATG-3'						
					Temperature (°C)		
α_{s2}-casein	5'-TATGACATGTCGAGAAATGAG-3'	–1150/–872	278		94	58	72
	5'-ATAGAAGATAAACTGGCACGA-3'			1–34	1.0	1.0	1.0
α_{s1}-casein	5'-TATGACATGTCGAGAAATGAG-3'	–1145/+101	1246				
	5'-TTGGAACAATGCTATTAGGTT-3'						

2.3. SDS-PAGE

Polyacrylamide gel electrophoresis, performed under denaturating conditions with 0.1% SDS, was used to separate milk proteins. Skimmed milk samples, α-, β- and κ-casein standards [34], and an α_{s2}-casein standard (received as a gift from Dr C.J. Slangen, Netherland Institute for Dairy Research, NIZO) were denatured by boiling them with 2-mercaptoethanol (Sigma-Aldrich Corporation, St. Louis, MO, USA). All SDS-PAGE protein analyses were carried out as described by Grosclaude et al. [14]. The gels were run overnight at 180 V. After electrophoresis, gels were stained overnight with Coomassie brilliant blue R-250. The stained proteins were scanned in the FX Molecular Imager (Bio-Rad Laboratories Inc., Hercules, CA, USA), and densitometry was performed using the Quantity One program.

2.4. HPLC

High performance liquid chromatography analyses were carried out at the Institute of Animal Breeding, at Warsaw Agricultural University. All chemicals and solvents were purchased from Sigma (Sigma-Aldrich Corporation, St. Louis, MO, USA). Skimmed milk samples were precipitated with 10% CH_3COOH at its isoelectric pH (4.6). Casein was dissolved in BIS-TRIS® buffer (pH 7.0) and filtered through 0.45 μm Millipore, Milex-XV filters (Milipore Corporation, Bedford, MA, USA). Proteins were fractionated and quantitatively analysed by HPLC, using a Hewlett Packard Agilent 1050 (Hewlett-Packard GmbH, Waldbronn, Germany). Reversed- phase columns (C_4 and C_{18}) were used. Elution was done with acetonitryle-water-trifluoroacetic acid solution. The flow rate ranged from 0.5 to 0.8 $mL \cdot min^{-1}$, and eluted casein peaks were detected at 214, 220 and 280 nm. Quantitative analyses were done with external standards purchased from Sigma, and with casein standards that were a gift (see 2.3).

2.5. Statistical analysis

Differences in casein content, in milk from cows carrying specific mutations in the casein gene promoters, were statistically evaluated with the least squares method using Harvey software [15].

The model used was:

$$y_{ij} = \mu + M_i + LS_j + e_{ij}$$

where: y_{ij}: casein content; μ: overall means; M_i: effect of mutation (i = 1, 2); LS_j: combined effect of lactation number and lactation stage (j = 1, 11); e_{ij}: random error.

Table II. Frequency of genotypes in 5'-flanking regions (promoters) of α_{s1}- and α_{s2}-casein genes in Polish Red and Black-and-White cattle.

Locus/position of polymorphic site	Genotype	Black-and-White cattle		Polish Red cattle	
		number of animals	allele frequency	number of animals	allele frequency
α_{s2}-casein	TT	60	T - 1.00	178	T - 0.97
–186	CT	0	C - 0.00	10	C - 0.03
		total: 60		total: 188	
α_{s2}-casein	CC	116	C - 0.86	173	C - 0.94
–1084	CT	41	T - 0.14	22	T - 0.06
	TT	1			
		total:158		total: 195	
α_{s1}-casein	(-/-)	49	(–) - 0.95	21	(–) - 0.88
–728	(T/-)	5	(T) - 0.05	7	(T) - 0.12
		total: 54		total: 28	

Occurrence of the common genotypes (haplotypes) was analysed by Chi-square testing.

3. RESULTS AND DISCUSSION

Restriction fragment length polymorphisms (RFLPs) of the bovine α_{s2}-, α_{s1}- and β-casein gene promoters were investigated at polymorphic sites previously reported by Schild and Geldermann [28]. In the α_{s2}-casein gene promoter, polymorphisms occurred at positions –1084 and –186, relative to the transcription start point. Both of them were C/T transitions. In the case of the –1084 polymorphic site, a 278-bp DNA fragment was amplified and digested with *Mae*II endonuclease. Three genotypes were observed: CC, showing one undigested fragment; TT with two fragments (212 bp and 66 bp), and CT with three fragments – 278 bp, 212 bp and 66 bp (not shown). The analyses covered 195 PR and 158 BW cows. The frequency of allele C was 0.86 for BW, and 0.94 for PR cattle (Tab. II). The TT genotype was not found amongst PR animals, and only one TT animal was found in the BW breed. The results showed that the C →T transition in the bovine α_{s2}-casein gene promoter at position –1084 was rare, at least in the BW and PR cattle breeds. Previously, Schild and Geldermann [28] reported this mutation in one Zebu (*Bos indicus*) animal and in one German Simmental only.

RFLP-*Eco*RV analyses of the polymorphic site at position –186, in the bovine α_{s2}-casein gene promoter, resulted in two restriction fragments (965 bp and 302 bp) for the TT genotype, whilst the CT genotype showed three fragments 1267 bp, 965 bp and 302 bp (not shown). The TT and CT genotypes were found amongst 188 PR cows but only the TT genotype was found within 60 BW animals (Tab. II). The frequency of allele T was 0.97 and 1.00 in PR and BW, respectively. The low frequency of allele C found in this study confirmed results reported earlier by Schild and Geldermann [28], who identified only two animals with allele C (in combination with T), from one Zebu and one German Simmental.

The mutation in the bovine α_{s1}-casein gene at position –728 was a deletion T/–. Digestion with *Ssp*I endonuclease showed two genotypes – (–/–) with two restriction fragments of 828 bp and 145 bp, and the (T/–) genotype with three fragments of 972 bp, 828 bp and 145 bp (not shown). The frequency of allele (–) was 0.95 and 0.88 for BW and PR cattle, respectively (Tab. II).

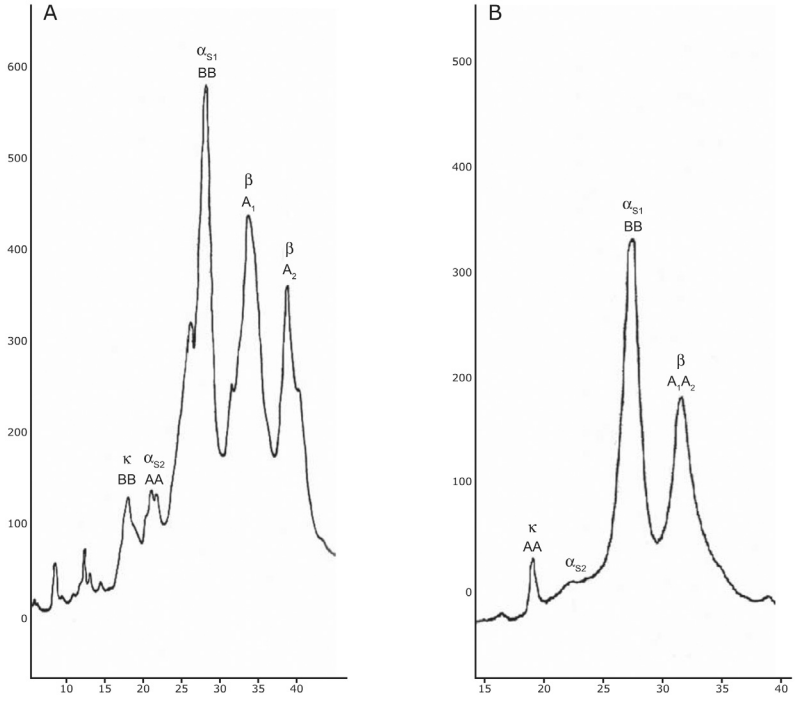

Figure 1. Chromatographic patterns (HPLC) of milk protein samples from two Black-and-White cows with CT (A) and CC (B) genotypes in the 5'-flanking region of the α_{S2}-casein gene at position –1084. The α_{S1}-casein is present as BB genotype, α_{S2}-casein is present as AA genotype (A) or apparently absent (B), β-casein – A_1A_2 or A_1A_1, and κ-casein – BB or BB genotype.

The T allele appeared slightly more frequently in PR than in BW cattle (0.12 vs. 0.05). The high frequency of allele (–) was also reported previously by Schild and Geldermann [28], who found only two TT animals amongst 13 animals belonging to 7 cattle breeds.

The C→ G transversion at position –109 in the 5'-flanking region of the β-casein gene can be recognised by the *Nla*III restriction nuclease, but was not found in this study, either in PC or in BW cattle. All analysed animals were CC homozygotes. This mutation was previously reported [28], but was found in only one animal out of thirteen tested.

3.1. Analysis of milk proteins

Genetic variants and the content of the calcium-sensitive caseins α_{S1}, α_{S2} and β in milk were analysed by SDS-PAGE and HPLC in cows carrying various genotypes within the α_{S1}- and α_{S2}-casein gene promoters. Milk samples were taken from 30 PR cows carrying either CT or TT genotypes in the α_{S2}-casein gene (position –186) and from 22 BW cows with either CC or CT genotypes (position –1084). Milk from both BW and PR cows was included in these studies, since both breeds differ in the frequency and distribution of α_{S2}-casein gene promoter genotypes, thus enabling the study of different combinations of gene variants between promoters and coding sequences. The results of representative milk protein separations carried out with SDS-PAGE and HPLC are shown in Figures 1–4.

Bovine α_{S1}-casein mostly occurs as genetic variants A or B with variant A differing from B by a deletion of 13 amino

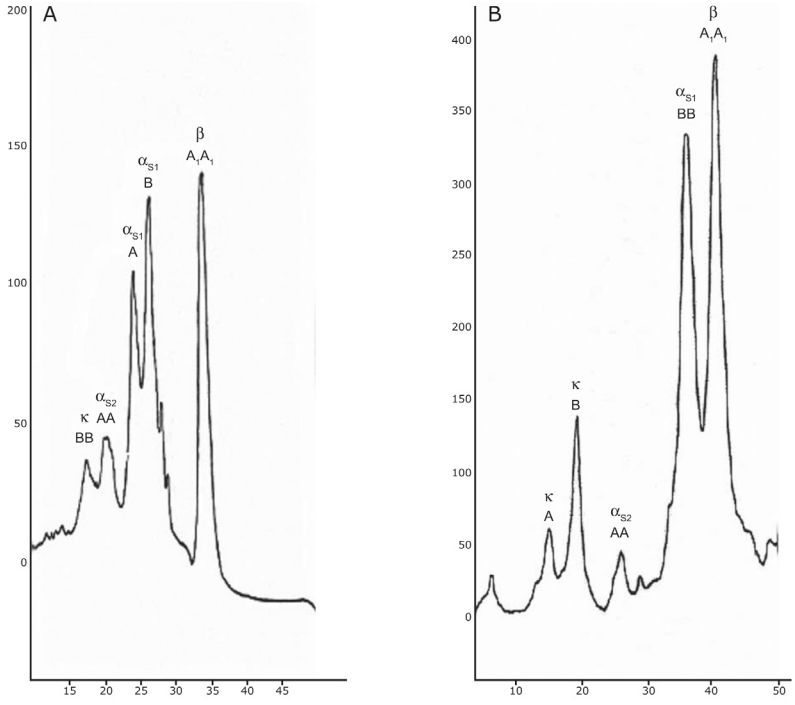

Figure 2. Chromatographic patterns (HPLC) of milk protein samples from two Polish Red cows with CT (A) and TT (B) genotypes in the 5'-flanking region of α_{s2}-casein gene at position –186. The α_{s1}-casein is present as AB or BB genotype, α_{s2}-casein – AA genotype, β-casein – A_1A_1, and κ-casein – BB or AB genotype.

acids (a.a.) from a.a. position 14 to 26 [11]. In polyacrylamide gel electrophoresis, allele B of α_{s1}-casein appeared as a slower migrating band in comparison with the fast migrating allele A (Figs. 3, 4). In HPLC, variant A of α_{s1}-casein was eluted earlier from the column than variant B (Figs. 1, 2). The most common variants of bovine β-casein are A1 and A2, differing by His→Pro substitution at a.a. position 67 [11]. The A_1A_1 genotype of β-casein appeared as a single slow migrating band, whereas A_1A_2 genotype variants migrated in two bands in SDS-PAGE (Figs. 3, 4), and showed two distinctly separated peaks in HPLC (Figs. 4, 5).

Numbers of genotypes and allele frequencies of α_{s1}- and β-casein in BW and PR cows, as determined by the use of SDS-PAGE and HPLC, are shown in Table III. In the case of α_{s1}-casein, allele B was mostly found with a frequency of 1.0 and 0.83 in BW and PR cattle, respectively. Known in the literature are five genetic variants of bovine α_{s1}-casein – A, B, C, D and E. In European cattle breeds, variant B is present at a very high frequency (0.9), and is highest in the Ayrshire breed (1.0). Allele B was also found predominantly in PR cattle, while variants C and D were rare [8, 9, 25].

The A_1 and A_2 alleles of β-casein existed mostly as A_1A_1 or A_1A_2 genotypes in this study (Tab. III). The frequency of allele A_1 was 0.77 and 0.79 in BW and PR cattle, respectively. Only one A_2A_2 BW individual was found among the 52 animals analysed. However, allele A_1 was slightly more frequent when compared with earlier reports

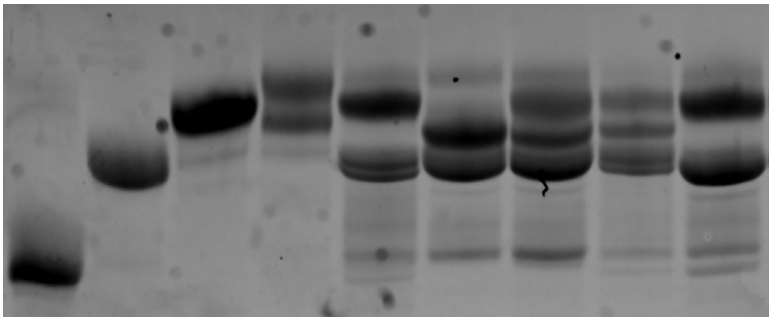

Figure 3. Polyacrylamide gel electrophoresis (SDS-PAGE) of bovine caseins. Milk samples were obtained from Polish Red cows with CT and TT genotypes of α_{s2}-casein gene promoter (position –186). Associations between the 5'-flanking variants CT and TT of the α_{s2}-casein gene and the casein variants are shown. Homozygous α_{s1}-casein genotype BB (lines 1 and 5), AA (line 2), and heterozygous AB (lines 3 and 4). Homozygous β-casein A_1A_1 (lines 2 and 5) and heterozygous A_1A_2 (lines 1, 3 and 4). κ-, β-, α_{s1}- and α_{s2}-cas are casein standards.

Table III. Milk protein genotypes and allele frequencies in Polish Red and Polish Black-and-White cattle.

Milk protein	Genotype	Black-and-White cattle		Polish Red cattle	
		number of animals	allele frequency	number of animals	allele frequency
α_{s1}-casein	BB	22	B - 1	20	B - 0.83
	AB	0	A - 0	10	A - 0.17
		total: 22	A	total: 30	
β-casein	A_1A_1	13	A_1 - 0.77	17	A_1 - 0.79
	A_1A_2	8	A_2 - 0.23	13	A_2 - 0.21
	A_2A_2	1			
		total: 22		total: 30	

for Polish, German and Danish Red cattle (0.61, 0.62, and 0.71, respectively; Michalak [25], Feleńczak [9], Litwinczuk [22], Erhardt [8]). In different populations of the Black-and-White cattle, variant A_1 of β-casein was found at a frequency of 0.36–0.72 [11].

In this study, only variant A of the α_{s2}-casein was found among PR and BW cattle. In SDS-PAGE, α_{s2}-casein migrated as a thin band seen at the front of α_{s1}-casein (Fig. 3). In HPLC diagrams, α_{s2}-casein formed a small peak migrating between κ- and α_{s1}-casein fractions (Figs. 1, 2). Interestingly, in several milk samples (derived from 4 BW and 4 PR cows) no detectable amounts of α_{s2}-casein were found (Fig. 1B). However, this phenomenon was apparently not associated with considered mutations within the α_{s2}-casein gene promoter (C/T at positions –1084 and –186), since milk from animals with various 5'-flanking region genotypes were represented. Four genetic variants of bovine α_{s2}-casein – A, B, C and D – are known. Only variant A was found in most European cattle breeds; variant C was found in yaks and variant B in *Bos indicus* (Zebu) [7]. In BW cattle, the frequency of variant A of α_{s2}-casein was 1.0 [11], while

Table IV. Expected and observed frequencies of intergenic haplotypes within bovine casein locus.

	PR cattle; α_{s2}-casein gene polymorphism (pos. –186)		
α_{s1}-casein	Genotype of α_{s2}-casein promoter		
		CT	TT
Allele A	Observed	11	0
	Expected	3.8	7.2
Allele B	Observed	9	38
	Expected	16.2	30.8
	BW cattle; α_{s2}-casein gene polymorphism (pos. –1084)		
β-casein	Genotype of α_{s2}-casein promoter		
		CT	CC
Allele A_1	Observed	14	18
	Expected	18.3	13.7
Allele A_2	Observed	10	0
	Expected	5.7	4.3

in Polish and German Red cattle variant A was predominant (0.98), and D was rare (0.02) [8].

Genotyping of caseins in individual animals showed significant associations between specific variants of α_{s2}-casein gene promoters and the genotypes of α_{s1}- and β-caseins; some combinations were much more frequent then others (Tab. IV). In PR cattle, significant association (Chi-square, $P \leq 0.001$) was found between variants in the 5'-flanking region (position –186) of the α_{s2}-casein gene and the α_{s1}-casein genotype. Allele A of α_{s1}-casein was associated with genotype CT in the α_{s2} gene promoter, whereas allele B was associated with genotype TT (Tab. IV; Fig. 3). In BW cattle, such association ($P \leq 0.02$) was found between allele A_2 of casein β and genotype CT in the α_{s2}-casein gene promoter (position –1084), whereas allele A_1 was associated with genotype CC (Tab. IV; Fig. 4).

Associations between polymorphism in the α_{s1}-casein gene promoter and α_{s1}-casein variants were not observed (Tab. V). The present results suggest the existence of intergenic haplotypes within the casein *locus*. Associations CT/A, TT/B and CT/A_2, CC/A_1 between genotypes of the α_{s2}-casein gene promoter and casein α_{s1} and β, respectively, is obviously a result of strong linkage of these genes and their common localisation on cattle chromosome 6 [10, 31].

Associations were searched for between polymorphisms in promoter regions of the α_{s2}- and α_{s1}-casein genes and casein contents in milk. SDS-PAGE gels with stained milk proteins were scanned and densitometry was performed. Moreover, quantitative analyses were done by HPLC.

Differences in α_{s2}-casein content were shown in milk derived from cows with different α_{s2}-casein gene promoter variants at positions –1084 and –186 (Figs. 5A, 5C). As shown in Figure 5A, the –1084 CT genotype is associated with a higher amount of α_{s2}-casein in milk, as compared with the CC genotype. The difference approached the significance of $P \leq 0.08$. Moreover, animals with the α_{s2}-casein CT genotype (–1084)

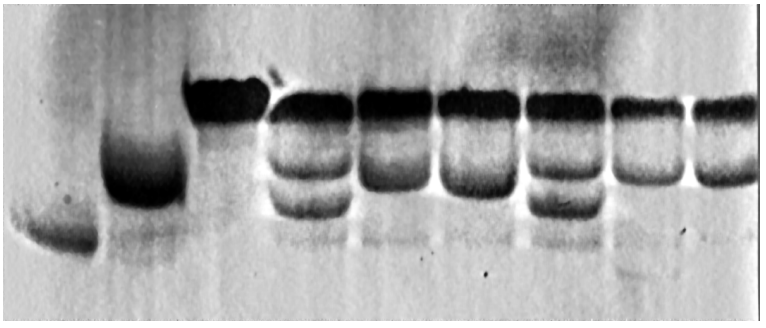

Figure 4. Polyacrylamide gel electrophoresis (SDS-PAGE) of bovine caseins. Milk samples were obtained from Black-and-White cows with CT and CC genotypes of the α_{s2}-casein gene promoter (position –1084). Associations between the 5'-flanking variants CT and CC of the α_{s2}-casein gene and casein variants are shown. Homozygous α_{s1}-casein genotype BB (lines 1–6), homozygous β-casein A_1A_1 (lines 2, 3, 5 and 6), and heterozygous A_1A_2 (lines 1 and 4). κ-, β- and α-cas are casein standards.

Table V. Expected and observed frequencies of intragenic haplotypes within bovine casein locus.

	PR cattle; α_{s1}-casein gene polymorphism (pos. –728)		
α_{s1}-casein	Genotype of α_{s1}-casein promoter		
		T/–	–/–
Allele A	Observed	3	8
	Expected	2.75	8.25
Allele B	Observed	11	38
	Expected	12.25	36.75

had statistically more β-casein in their milk ($P \leq 0.01$; Fig. 5B). The second C/T transition in the α_{s2}-casein gene promoter (position –186) was associated with α_{s2}-casein content. Cows with the TT genotype had more α_{s2}-casein in their milk than CT heterozygotes ($P \leq 0.07$; Fig. 5C).

Ehrmann et al. [6] detected differences in the expression of milk proteins in the mammary glands of cows with variants in the 5'-flanking regions of the β-lactoglobulin gene. Association has also been described between alleles in coding regions of the milk protein genes, and the levels of their expression in milk [32]. This is possibly caused by linkage between variants of coding and regulatory regions. Black and Bremel [2] suggested that the point mutation at position +15 in the α-lactalbumin gene (region coding for 5' UTR) was a direct cause of the differences in milk production, and proposed this polymorphism as being a quantitative trait *locus* (QTL) on chromosome 5 of dairy cattle. Lum et al. [23] studied differential expression of two common allelic variants of β-lactoglobulin – A and B. The authors hypothesised that the G to C substitution at position –430 is a potential candidate for allele-specific regulation of β-lactoglobulin expression by interfering with binding of the AP-2 transcription factor. Kuss et al. [19] have reported the association of a single nucleotide polymorphism in the β-lactoglobulin gene promoter (G or C

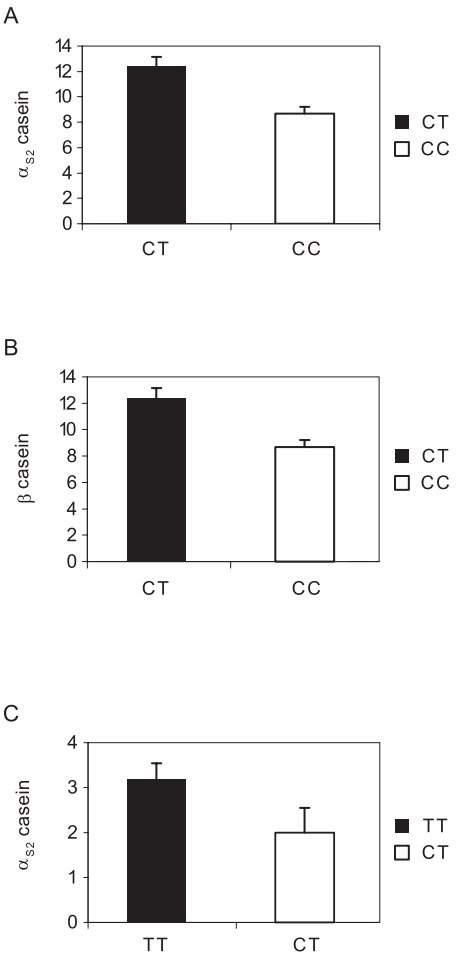

Figure 5. Analysis of α_{s2}- and β-casein contents in cow's milk with SDS-PAGE and HPLC. Quantitative differences in casein contents were expressed in arbitrary units. Six cows representing different casein promoter genotypes were used in every experiment; values represent the average (LSM ± SE). Milk was obtained from 3 Black-and-White cows with CC genotype and 3 with CT genotype in the α_{s2}-casein gene promoter (position –1084) and the content of α_{s2}-casein **(A)** or of β-casein **(B)** was measured (combined PAGE/HPLC data). **(C)** The α_{s2}-casein content in milk from Polish Red cows with TT and CT genotypes of the α_{s2}-casein gene promoter (position –186).

at position –435) with amounts of β-lactoglobulin in milk. They also demonstrated the association between single nucleotide polymorphism (SNP) in the β-lactoglobulin gene promoter, and amounts of α-lactalbumin and α_{s1}-, β- and κ-caseins. The association between the SNP in the AP-2 binding site of the β-lactoglobulin gene and its gene product can be explained as a result of differences in protein binding capacity to DNA, and allele-specific differences in gene expression. Geldermann et al. [12] showed that, in a cell culture model, variant AA of the β-lactoglobulin gene promoter produced up to 3.5 times higher reporter gene expression than the BB genotype of β-lactoglobulin. Recently, Cardak et al. [5] have reported that genotypes of milk protein coding gene loci had a significant effect on contents, as well as yields, of corresponding milk proteins – α_{s1}-, β- and κ-casein and β-lactoglobulin – in the milk of Holstein-Friesian and Simmental cows.

A number of groups [2, 28, 29] have hypothesised that inheritable variations in nucleotide sequences in gene regulatory elements might lead to differences in transcription rates, by decreasing or increasing the abundance of specific mRNAs. Thus, this would lead to differences in the amount of relevant proteins produced in cow's milk.

As we showed previously, nucleotide substitutions in the 5'-flanking region of the bovine casein genes affect binding of transcription factors [30]. The C/T transition in the α_{s2}-casein gene promoter appeared to influence the casein gene transcript levels. More α_{s2}-casein gene transcripts were found in the RNA isolated from the mammary gland tissue genotyped as the α_{s2}-casein CT genotype than the CC genotype. In this study, we showed an association of sequence nucleotide polymorphisms in the 5'-flanking region of the bovine α_{s2}-casein gene with α_{s2}- and β-casein contents in milk. In particular, cows carrying CT genotypes at the α_{s2}-casein gene promoter contained more α_{s2}-casein in milk. Thus, a mutation in the gene regulatory region might affect the levels of gene products in

milk. However, it must be stressed that our study only showed associations of certain SNPs with protein isoforms and content in milk, but provided no direct evidence for a causative relationship. It cannot be excluded that other mutations, not studied here, contributed to the differences seen in the levels of proteins in milk. Nevertheless, we hypothesise that these changes might be caused by alterations in affinity between transcription factors and promoters, these being the proteins principally involved in gene transcription regulation.

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