\(\alpha_{\text{S1}}\)-casein yield and milk composition are associated with a polymorphic regulatory element in the bovine \(\alpha_{\text{S1}}\)-casein gene

A.W. Kuss\(^1\), T. Peischl\(^1\), J. Gogol\(^1\), H. Bartenschlager\(^1\) & H. Geldermann\(^1\)

\(^1\) Department of Animal Breeding and Biotechnology, University of Hohenheim, D-70593 Stuttgart, Germany

Summary

In this study the protein synthesised from the bovine \(\alpha_{\text{S1}}\)-casein gene was to be quantified and investigated in view of associations with DNA-variants in the 5'-flanking region. Blood and milk samples from cows of the breeds German Holstein Friesian and Simmental were analysed. Isolelectric focussing was used for allelic identification of polymorphic milk proteins, and alkaline Urea-PAGE in combination with densitometry was applied for quantification of milk protein fractions (\(\alpha\)-lactalbumin, \(\beta\)-lactoglobulin, \(\alpha_{\text{S1}}\), \(\beta\)- and \(\kappa\)-casein) in at least four milk samples per cow. DNA variants were analysed in the 5'-flanking region of the \(\alpha_{\text{S1}}\)-casein encoding gene by PCR-RFLP. The findings reveal associations with the relative proportion as well as the yield of \(\alpha_{\text{S1}}\)-casein. The results represent an example for a potentially specific influence of variable regulatory elements on gene expression and gene product quantities, therefore being of relevance for further breeding on milk protein yield and composition.

Keywords: AP-1, \(\alpha_{\text{S1}}\)-casein, cattle, polymorphism, promoter

Introduction

\(\alpha\)-s1 casein (\(\alpha\)-s1 CN) is with a proportion of 35 -45 % the major contributor to the protein fraction of bovine milk. It’s encoding gene (CSN1S1) is part of the casein cluster, located on BTA 6 and paralog with the \(\alpha_{\text{S2}}\) and \(\beta\)-casein encoding genes (for review see e. g. Rijnkels, 2002).

DNA variants occur in the coding region of the CSN1S1 (Mercier et al., 1971; Grosclaude et al., 1972; Grosclaude et al., 1970; Prinzenberg et al., 1998) as well as the non-coding areas such as the promoter region (Schild & Geldermann, 1996). Variants of the coding region of bovine CSN1S1 and their associations with quantitative effects on milk performance parameters have hitherto been investigated abundantly (e.g. McLean et al., 1984; NG-Kwai-Hang et al., 1987; Ehrmann et al., 1997b).

Also for polymorphisms in the 5'-flanking region of milk protein encoding genes observations showing significant associations with relative content as well as amount of single milk proteins have been made (Ehrmann et al., 1997b, for review see Martin et al. 2002). When regarding the bovine \(\beta\)-lactoglobulin encoding gene, we have already been able to find associations of a variable AP-2 element with relative content as well as amount of the gene product which strongly suggest being due to allelic differences in gene expression (Kuss et al., 2003).
The study presented here was designed as a population analysis for milk protein traits in association with a polymorphic putative regulatory element in the promoter region of CSN1S1. This element comprises an A to G SNP (Schild & Geldermann, 1996) affecting the 5' end of an AP-1 consensus sequence (TGAGTCTA; Vogt & Bos, 1990; Angel & Karin, 1991).

Material and methods

Animals and samples

Samples and data were collected between April 1997 and March 1999 from 80 cows of a German Holstein Friesian (HF) herd and 62 cows of a Simmental (SM) herd, both from experimental stations of the Hohenheim University. For pedigree information see Kuss et al. (2003). At least four milk samples per cow were collected in intervals of eight weeks during lactation and defatted samples were stored at –80 °C. Genomic DNA was isolated from blood by means of the NucleoSpin® Blood Quick Pure kit (Macherey Nagel, Düren, Germany) according to the instructions of the manufacturer.

Quantification of Milk Proteins

Milk proteins were separated by vertical Urea-PAGE (8 M Urea, 8.4 % Acrylamide; see Ehrmann et al., 1997a). The gels were then stained (staining buffer: 8 % w/v Trichloroacetic acid, 26.7 % v/v Methanol, 9.3 % v/v Acetic Acid, 0.033 % w/v Coomassie Brilliant Blue R250) overnight with agitation. After standardized destaining (29 % v/v Methanol, 5 % v/v Acetic Acid; 4 h with agitation), milk protein fractions (α-s1-Casein, β-Casein, κ-Casein, α-Lactalbumin, β-Lactoglobulin) were quantified using a densitometer (ATH Elscript 400, Hirschmann, Germany) with software of the manufacturer.

Genotyping of DNAVariants and Statistical Analysis

A 192 bp fragment of the bovine CSN1S1 5’-flanking region between -317 bp and -125 bp was amplified by PCR from genomic DNA. The amplicon was digested with NmuCl (MBI Fermentas, St.Leon-Rot, Germany) and subsequently submitted to agarose gel analysis for the RFLP (restriction fragment length polymorphism) determination of the polymorphic AP-1 site R1.3 (SNP at -175 bp, described by Schild & Geldermann, 1996). Genotype nomenclature refers to the respective nucleotide (A or G) at Position –175 bp. Statistical analysis was carried out as previously described (Kuss et al., 2003).

Results

Homozygotes for the G-allele were not observed, A-homozygotes occurred at a four fold frequency as compared to heterozygotes and the allele frequencies of the G-allele were 0.05 in HF and 0.16 in SM.

The results of an analysis across both breeds are presented in Table 1, showing that the carriers of the G-variant were significantly superior both in yield (g/day) as well as content (% of protein fraction) of α-s1-Cn as compared to A-homozygotes.
Table 1: Associations between content (Percentage of protein fraction) and amount (g/day) of α_s1-CN and genotypes of the variable AP1 binding site (-175bp) in the promoter region of CSN1S1

<table>
<thead>
<tr>
<th>Genotype (Frequency)</th>
<th>Number of animals</th>
<th>Percentage of protein fraction LSQ mean ± SE</th>
<th>Amount of protein [g/day] LSQ mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (0.8)</td>
<td>114</td>
<td>36.76^a ± 0.12</td>
<td>258.44^a ± 2.38</td>
</tr>
<tr>
<td>AG (0.2)</td>
<td>28</td>
<td>40.08^b ± 0.28</td>
<td>294.99^b ± 5.29</td>
</tr>
</tbody>
</table>

P^2: ***

[^1] Average for both breeds;[^2] ***: p < 0.001 (F-Test); Significant differences (p<0.01; t-Test) between genotypes are indicated by different letters; ***: p < 0.001 (F-Test)

The content of β-CN and κ-CN was lower in the milk of homozygous carriers of the A-allele in both breeds (not shown). Concerning the amount of β-CN and κ-CN the observed differences did not reach the level of significance. The percentage of total protein was 3.41 ± 0.01 for homozygotes as compared to 3.29 ± 0.02 for heterozygotes (p<0.001). The whey proteins β-Lactoglobulin and α-Lactalbumin did not show associations in SM, while in HF an increase (p< 0.001) in content and amount of α-Lactalbumin appeared to be associated with the G-allele (not shown). This was accompanied by a significantly enhanced total protein yield while the total protein content (% of whole milk) in HF carriers of the G-allele was reduced (p< 0.001).

Discussion

In view of previous findings for β-Lactoglobulin (Lum et al., 1997; Kuss et al., 2003), as well as for promoter variants in CSN1S1 and CSN1S2 (Martin et al., 2002) the differences we observed between carriers of the AP-1 alleles A and G concerning yield and amount of α_s1-CN indicate differential protein binding properties of the variable promoter element. This was confirmed by EMSA results (not shown) that revealed reduced protein binding of oligos containing the G-variant which represents only about 87% of the AP-1 consensus sequence. Hence our findings suggest that the involved AP-1 factor functions as a repressor for the expression of α_s1-CN and that therefore an impaired consensus sequence can be conducive to enhanced α_s1-CN expression. This interpretation is corroborated by the observation that c-jun, the major component of AP-1 dimers (for review see e. g. Karin et al., 1997), plays a role in cell proliferation and survival also as a repressor rather than an enhancer (reviewed by Shaulian & Karin, 2001). The presence of AP-1 factors in mammary epithelial cells and their involvement in mammary gene regulation have been established e. g. by Olazabal et al. (2000).

Our findings as to differences in total protein content between A-homozygotes and heterozygotes in HF are in agreement with the observations of Prinzenberg et al. (2003) who, by analysis of variance using a granddaughter design, found associations between SSCP genotypes of the CSN1S1 promoter and protein percentage in Holstein cattle. In Finnish
Ayrshire cows, QTL for both total protein percentage and yield have been assigned to the location of CSN1S1 (MARC97: 82.6 cM; Maki-Tanila et al., 1998 Ikonen et al., 1999).

Interestingly, the increased total protein yield we observed in HF coincided with decreased protein content of milk from carriers of the G-allele. This can be explained by the approximately 35% higher amount (g/day) of α-Lactalbumin (not shown) in the milk from these animals. Since α-Lactalbumin enhances the concentration of lactose which is the major osmol in milk (e.g. Ramakrishnan et al., 2001) its elevated production is likely to cause a higher water ratio. As these observations were not made in SM, we assume that they represent coincidental effects of breeding on high milk performance in HF.

Associations between CSN1S1 exon variants and αs1-CN traits which were previously observed in the same breeds e.g. by Cardak et al. (2003) might be due to the very close linkage of these loci with the AP-1 variant and therefore can, in accordance with suggestions by Koczyn et al. (1993) and Ehrmann et al. (1997a) imply the presence of “intragenic haplotypes”. The same assumption can be made with regard to the impact of specific milk proteins on the quality of milk such as the implication of CSN1S1 exon variants in superior protein and casein content (reviewed by Buchberger & Dovc, 2000). For cheese making ability traits however, polymorphisms affecting protein characteristics might have direct effects, independent of promoter variants.

Still, the observed close association between differential binding capacity of a potentially functional polymorphic promoter element in CSN1S1 and quantitative characteristics of the gene product requires further supporting analyses, e.g. reporter gene studies, in order to reveal additional evidence as to the true causal coherences.

References

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