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α_{S1} -casein yield and milk composition are associated with a polymorphic regulatory element in the bovine α_{S1} -casein gene

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Summary

In this study the protein synthesised from the bovine α_{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. Isoelectric 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 (α -lactalbumin, β -lactoglobulin, α_{S1} -, β - and κ -casein) in at least four milk samples per cow. DNA variants were analysed in the 5'-flanking region of the α_{S1} -casein encoding gene by PCR-RFLP. The findings reveal associations with the relative proportion as well as the yield of α_{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, α_{S1} -casein, cattle, polymorphism, promoter*

Introduction

α -s1 casein (α -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 α_{S2} - and β -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 β -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) over night 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 DNA Variants 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 *Nmu*CI (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

Genotype (Frequency) ¹	Number of animals	Percentage of protein fraction LSQ mean $\pm$ SE	Amount of protein [g/day] LSQ mean $\pm$ SE
AA (0.8)	114	36.76 ^a $\pm$ 0.12	258.44 ^a $\pm$ 2.38
AG (0.2)	28	40.08 ^b $\pm$ 0.28	294.99 ^b $\pm$ 5.29
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 osmole 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 α_{s1} -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 Koczan 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

- Angel, P. & M. Karin, 1991. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim. Biophys. Acta* 1072: 129-157
- Buchberger, J. & P. Dovc, 2000. Lactoprotein Genetic Variants in Cattle and Cheese Making Ability. *Food technol. biotechnol.* 38: 91-98
- Cardak, A. D., H. Bartenschlager, and H. Geldermann. 2003. Effects of polymorph milk proteins on the individual milk protein content of Holstein-Friesian and Simmental cows. *Milchwissenschaft* 58: 235-238
- Ehrmann, S., H. Bartenschlager, & H. Geldermann, 1997a. Polymorphism in the 5' flanking region of the bovine lactoglobulin encoding gene and its association with beta-lactoglobulin in the milk. *J. Anim. Breed. Genet.* 114: 49-53
- Ehrmann, S., H. Bartenschlager, & H. Geldermann, 1997b. Quantification of gene effects on single milk proteins in selected groups of dairy cows. *J. Anim. Breed. Genet.* 114: 121-132
- Grosclaude, F., M. F. Mahe, J. C. Mercier, & B. Ribadeau-Dumas, 1970. The A variant of bovine alpha(s1) casein is devoid of the segment of 13 amino acid residues which occupies the 14th to 26th position from the NH(2)-terminal in the polypeptide chain (198 residues) of the B and C variants. *FEBS Lett.* 11: 09-112
- Grosclaude, F., M. F. Mahe, J. C. Mercier, and B. Ribadeau-Dumas, 1972. Characterization of genetic variants of a S1 and bovine caseins. *Eur. J. Biochem.* 26: 328-337
- Ikonen, T., M. Ojala, & O. Ruottinen, 1999. Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows. *J. Dairy Sci.* 82: 1026-1033
- Karin, M., Z. Liu, & E. Zandi, 1997. AP-1 function and regulation. *Curr. Opin. Cell Biol.* 9: 240-246

- Koczan, D., G. Hobom, & H. M. Seyfert, 1993. Characterization of the bovine alpha S1-casein gene C-allele, based on a MaeIII polymorphism. *Anim Genet.* 24: 74
- Kuss, A. W., J. Gogol, & H. Geldermann, 2003. Associations of a polymorphic AP-2 binding site in the 5'-flanking region of the bovine beta-lactoglobulin gene with milk proteins. *J. Dairy Sci.* 86: 2213-2218
- Lum, L. S., P. Dovc, & J. F. Medrano, 1997. Polymorphisms of bovine beta-lactoglobulin promoter and differences in the binding affinity of activator protein-2 transcription factor. *J. Dairy Sci.* 80: 1389-1397
- Maki-Tanila, A., D. J. de Koning, K. Elo, S. Moisio, & R. Velmala, 1998. Mapping of multiple quantitative trait loci by regression in half sib designs. *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, Armidale, Australia.* 25: 269-272.
- Martin, P., M. Szymanowska, L. Zwierzchowski, & C. Leroux, 2002. The impact of genetic polymorphisms on the protein composition of ruminant milks. *Reprod. Nutr. Dev.* 42: 433-459
- McLean, D. M., E. R. Graham, R. W. Ponzoni, & H. A. McKenzie, 1984. Effects of milk protein genetic variants on milk yield and composition. *J. Dairy Res.* 51: 531-546
- Mercier, J. C., F. Grosclaude, & B. Ribadeau-Dumas, 1971. Primary structure of bovine s1 casein. Complete sequence. *Eur. J. Biochem.* 23: 41-51
- Ng-Kwai-Hang, K. F., J. F. Hayes, J. E. Moxley, & H. G. Monardes, 1987. Variation in milk protein concentrations associated with genetic polymorphism and environmental factors. *J. Dairy Sci.* 70: 563-570
- Olazabal, I., J. Munoz, S. Ogueta, E. Obregon, & J. P. Garcia-Ruiz, 2000. Prolactin (PRL)-PRL receptor system increases cell proliferation involving JNK (c-Jun amino terminal kinase) and AP-1 activation: inhibition by glucocorticoids. *Mol. Endocrinol.* 14: 564-575
- Prinzenberg, E. M., P. Anglade, B. Ribadeau-Dumas, & G. Erhardt, 1998. Biochemical characterization of bovine alpha s1-casein F and genotyping with sequence-specific primers. *J. Dairy Res.* 65: 223-231
- Prinzenberg, E. M., C. Weimann, H. Brandt, J. Bennewitz, E. Kalm, M. Schwerin, & G. Erhardt, 2003. Polymorphism of the bovine CSN1S1 promoter: linkage mapping, intragenic haplotypes, and effects on milk production traits. *J. Dairy Sci.* 86: 2696-2705
- Ramakrishnan, B., P. S. Shah, & P. K. Qasba, 2001. alpha-Lactalbumin (LA) stimulates milk beta-1,4-galactosyltransferase I (beta 4Gal-T1) to transfer glucose from UDP-glucose to N-acetylglucosamine. Crystal structure of beta 4Gal-T1 x LA complex with UDP-Glc. *J. Biol. Chem.* 276: 37665-37671
- Rijnkels, M., 2002. Multispecies comparison of the casein gene loci and evolution of casein gene family. *J. Mammary. Gland. Biol. Neoplasia.* 7: 327-345.
- Shaulian, E. & M. Karin, 2001. AP-1 in cell proliferation and survival. *Oncogene* 20: 2390-2400
- Schild, T. A. & H. Geldermann, 1996. Variants within the 5'-flanking regions of bovine milk-protein-encoding genes. III. Genes encoding the Ca-sensitive Caseins alpha-s1, alpha-s2 and beta. *Theor.Appl.Genet.* 93: 887-893
- Vogt, P. K. & T. J. Bos, 1990. jun: oncogene and transcription factor. *Adv. Cancer Res.* 55: 1-35