EFFECT OF POLYMORPHISMS IN THE *CSN1S1* GENE ON MILK PRODUCTION TRAITS IN GERMAN HOLSTEINS

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INTRODUCTION

Effects of milk protein polymorphisms on milk production traits have been investigated by different groups and results are still somewhat contradictionary (e.g. Aleandri *et al*. 1990 ; Bovenhuis *et al*., 1992 ; Ng-Kwai-Hang *et al*., 1990). Depending on breed or region the same genes and alleles were associated with different traits and / or effects. One reason for these contradictions could be a set of different intragenic haplotypes comprising distinct variants in the regulatory elements combined with the same protein variant expressed in milk (Ehrmann *et al*., 1997). Scans for QTLs on BTA6 including microsatellites and the casein genes as markers indicate possible QTls close to the casein locus region (Bovenhuis and Weller, 1994 ; Freyer *et al*., 1995 and 2002, Velmala *et al*., 1999). *CSN1S1* is nearly invariant at protein level in Holstein Friesians with frequencies for *CSN1S1*B* varying from 0.872 in Finnish (Lien *et al*., 1999), to 0.985 in Canadian and German Holsteins (Ng-Kwai-Hang *et al*., 1990, Erhardt; 1993). Different *CSN1S1* promoter polymorphisms were described by Schild and Geldermann (1996) for various breeds including Holsteins. Comparisons of two *CSN1S1* promoter types as described by Koczan *et al*. (1993) and the two alleles *CSN1S1*B* and *C* of the protein coding region revealed up to four haplotypes depending on the breed investigated. In highly selected breeds as Angler and Ayrshire only one or two haplotypes were found (Jann *et al*., 2002). The aim of our present study was to determine the variability in the proximal *CSN1S1* promoter region in German Holstein cows and evaluate possible effects of found polymorphisms on milk production traits.

MATERIAL AND METHODS

DNA samples and data collection. Individual DNA samples of about 500 German Holstein cows under official milk recording were used for allele frequency determinations. Milk yield and content trait data were collected and provided by "Vereinigte Informationssysteme Tierhaltung" (VIT) at Verden, Germany. In a second approach, DNA from a paternal half sib family belonging to a granddaughter design from the German QTL project was used.

Polymorphism detection. A 655 bp fragment from the proximal *CSN1S1* promoter was PCR amplified using primers selected from the *CSN1S1* sequence (Koczan *et al*, 1991 ; GenBank Accession No. X59856) in 15µl reactions containing 50-150ng genomic DNA, 10pmol of each primer, 0,5 U Taq DNA-polymerase (Peqlab Biotechnologie, Erlangen), 50µM dNTPs in standard reaction buffer (10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$) over 30 cycles: 1 min- 93°C (1x), 40 sec. – 91°C, 40 sec. 57°C, 40 sec – 70°C (30x) and a final 3 min step at 70°C. After completion of the PCR reaction, fragments were subjected to single

strand conformation polymorphism (SSCP) analysis. 25 µl of denaturing loading dye (95% formamide) were added, the 96-well plate was heated to 93°C for 2 min and chilled on ice immediately. 4µl of the denatured samples were loaded on a 16x16 cm 12% non denaturing polyacrylamide gel containing 1% glycerol and were separated overnight at 420V – 10°C for 20 hours and silver stained following standard protocols.

Statistical analyses. Allele frequencies for the promoter polymorphism were determined by direct counting. Rare genotypes that were not equally distributed over the farms were excluded for the variance component analyses. Variance component analysis was performed using SAS program package. The first lactation results of 439 cows with milk yield, fat and protein kg (421 additionally with fat and protein content) were analysed with a model including the fixed effects year of calving (1990 to 1997), farm (1 to 5) and genotype (12, 22, 23, 24 and 33). A half sib family of 54 bulls with de-regressed proofs as described by Thomsen et al. (2001) for milk yield, fat content, fat kg, protein content and protein kg based on a total of 38759 daughter records were analysed with the type of *CSN1S1* promoter inherited from the grandsire as fixed effect only.

RESULTS AND DISCUSSION

SSCP analysis revealed four different patterns that were named 1, 2, 3 and 4 in the order of increasing mobility. Type 1 most likely corresponds to the type *c* as described by Koczan et al. (1993). Allele frequencies were 0.031 (type 1), 0.739 (type 2), 0.194 (type 3) and 0.036 (type 4). Genotype frequencies declined in the order 22 > 23> 24 > 12 > 33 > 34. No cows homozygous for 11, 44 or a combination of these rare alleles were found.

Variance component analysis for individual milk, fat and protein yields proved significant effects for farm and calving year, but no significant effects of the promoter polymorphisms were found. Nevertheless, the group of cows heterozygous for type 24 in tendency showed lower least square means for milk, fat and protein yield than groups without type 12, 22, 23, 33 (Table 1).

Table 1. Least square means (LSMEAN) for first lactation milk (MY1), fat (FY1) and protein (PY1) yield in cow groups with different *CSN1S1* promoter types

CSN1S1		LSMEAN for 305d yield		
promoter type	n	MY1 (kg)	FY1 (kg)	PY1 (kg)
12	24	5838.68	236.45	193.29
22	247	5884.67	238.21	194.40
23	121	5840.41	233.57	192.24
24	27	5556.69	226.00	184.30
33	20	5775.96	229.85	191.71

In the half-sib family from the granddaughter design, type 24 was segregating from the grandfather to his sons. Least square means for the de-regressed proofs for yield traits in the two groups inheriting type 2 or type 4 from the grandsire are shown in table 2.

Effect of type 4 on de-regressed proofs for milk yield (DRG_MY1), fat yield (DRG-FY1) and protein percentage (DRG_PP1) was significant (p = 0.035, 0.014 and 0.045). For DRG_PP1 effect of type 4 was positive, but because of opposite values for milk yield the effects for total protein yield were not significant anymore. On the other hand, no significant effects of the promoter type on de-regress proofs for fat percentage were found, thus DRG_FY1 is mainly dependent on DRG_MY1.

Table 2. Least square means for de-regressed proofs for milk yield (DRG_MY1), fat yield (DRG_FY1) and protein yield (DRG_PY1) in bulls inheriting promoter type 2 or 4 from their father

Paternal		LSMEAN ± se		
CSN1S1 type	n	DRG_MY1	DRG_FY1	DRG_PY1
4	29	57.820 ± 22.745*	2.228 ± 0.708*	2.569 ± 0.610
2	25	130.096 ± 24.497*	4.868 ± 0.763*	3.928 ± 0.657

* effects of type 4 proved significant (p < 0.05)

Results from the family support the initial tendency found in the individual cows, which also indicated lower milk yields associated with type 4. Fat and protein yield in the cows are directly dependent on milk yield and show the same directions. Previous studies postulated QTLs for milk and fat yield (Bovenhuis and Weller, 1994 ; Velmala *et al*., 1999) and for milk and fat yield, fat content (Freyer *et al*., 1995) or additionally protein content close to the casein locus (Freyer *et al*., 2002) which is in accordance with our findings.
If a direct gene effect is assumed, effects of a casein promoter on protein content would be expected. Nevertheless our results indicate only marginal - but significant - effects on this trait, leaving the question of a closely linked gene instead of a direct effect still open. Investigations in other granddaughter families are in progress to evaluate as well effects of promoter types 1, 2 and 3 as effects of type 4 in other families. This may either strengthen or reject the hypothesis of direct *CSN1S1* associated effects on milk production traits.

CONCLUSION

Our study confirms, that the casein locus is still an interesting candidate locus for milk yield and content traits. In contrast to most previous studies, we focused on the presumed regulatory region of the *CSN1S1* gene, which is in the highly selected population of German Holsteins still polymorphic. This circumvents one problem of previous studies, the impossibility of estimating effects of a virtually invariant gene. Further analyses will be necessary to clarify if the estimated effects are due to direct gene effects or linked loci.

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