THE USE OF BOVINE CASEIN HAPLOTYPES AS GENETIC MARKERS

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SUMMARY

The appropriateness of casein alleles assembled in haplotypes as genetics markers is studied. Polymorphic Information Content (PIC) of casein haplotypes (.78) is substantially higher than PIC of individual casein loci (ranging from .16 to .50) in Norwegian Cattle. Preliminary results show that haplotype 5 (with allele C of α_{s1}-CN) is significantly associated with high protein yield, protein percentage and milk yield. Haplotypes 7, 8, and 9 (with allele B of κ-CN) showed no significant associations with milk production traits. These results are compared to other studies in a literature review. The use of haplotype information in the genetic improvement of Norwegian Cattle is discussed.

INTRODUCTION

There is world wide interest in localising the loci responsible for milk production traits. A large number of studies have focused on the four casein genes (α_{s1}-CN, α_{s2}-CN, β-CN, and κ-CN), which constitute about 80% of the protein content in cow's milk. The casein genes are specifically expressed in the epithelial cells of the mammary gland during lactation, and are under complex multi-hormonal control (Vonderhaar & Ziska, 1989).

Most studies examining the associations between casein alleles and milk production traits treat the milk protein loci individually and test the alleles statistically for direct effects of each genotype across families. Alternatively, the alleles can be analyzed as markers within families, which means that also linked variation can be detected.

Most bovine casein alleles differ from one another by few base substitutions causing one or two amino acid changes in the protein (Eigel et al., 1984). The change of a few amino acids might change the physical and chemical properties of milk, but it seems unreasonable to suggest that they are responsible for the different levels of milk protein content. However, association between casein alleles and milk protein production could occur if there is linkage disequilibria between mutations in the coding regions and in the regulatory sequences of casein genes.

The tight genetic linkage of bovine casein loci permits the assembly of polymorphism in haplotypes, which can be considered as different alleles at a multi-allelic locus. Casein haplotypes have a higher polymorphic information content (PIC) than individual loci facilitating potential detection and further practical implantation in breeding programs.

The objective of this paper is to report on the suitability and potential uses of casein alleles assembled in haplotypes as genetic markers.

CASEIN HAPLOTYPES AS GENETIC MARKERS

A marker is suitable, in association studies with QTL, if the following conditions are met: a) rapid and cheap assay of genotypes; b) the marker generates high PIC; and c) linkage disequilibrium between alleles at the marker and the QTL.

The casein loci are physically mapped within a 200 kb fragment on bovine chromosome 6 (Feretti et al., 1990; Threadgill & Womack, 1990). Genetic linkage results, estimated by single sperm typing, show a tight genetic linkage among casein loci (Lien et al., 1993). Single sperm

typing is based on the isolation of single sperm cells and subsequent amplification from one copy of DNA by polymerase chain reaction (PCR). Each sperm cell represents a meiotic event and there are no limitations in the number of haploid cells to be analyzed from an individual. Lien et al. (1993) observed no recombinants in the analysis of 330 single sperm cells for three bovine casein loci, giving lod scores at zero recombination frequency ($\theta = 0$) of more than 32.

Casein alleles are relatively fast and inexpensively typed by PCR based methods (Medrano & Aguilar-Cordova, 1990; Lien et al., 1992). The analysis of 306 sons and 15 sires for casein polymorphisms revealed 10 different casein haplotypes in the Norwegian Cattle population (Lien & Rogne, 1993). Although many casein alleles have been identified, many of them are at a low frequency and yield a low PIC for each locus. The assembling of casein alleles in haplotypes generates a higher PIC than individual casein loci as shown in Table 1 for the Norwegian Cattle population.

Table 1. PIC values of casein polymorphisms in Norwegian Cattle[a].

	Polymorphisms			PIC
α_{s1}-CN	B, C			.16
β-CN	A1, A2, A5, B			.50
κ-CN	A, B, E			.27
κ-CN mikrosat.	14, 16			.38
Haplotypes[b]	1. B-A1-14-A	5. C-A5-14-A	9. B-B-14-B	.78
	2. B-A1-16-A	6. C-A5-16-A	10. B-A1-16-E	
	3. B-A2-14-A	7. B-A1-14-B		
	4. B-A2-16-A	8. B-A2-14-B		

[a]According to (Lien & Rogne, 1993).
[b]The casein haplotypes are arranged according to their position on the bovine chromosome 6 in the following order; α_{s1}-CN (B, C), β-CN (A1, A2, A5, B), a microsatellite in intron III of κ-CN (14 and 16 repeats) and κ-CN (A, B, E).

One important requirement for a marker is to have alleles in linkage disequilibrium with alleles at QTL affecting production traits. This can be tested by studying the associations between production traits and marker alleles. Associations between casein haplotypes and milk production traits using a granddaughter design in Norwegian Cattle is currently under investigation. One analysis was carried out using five bull sires with haplotype 5 (containing the C allele of α_{s1}-CN). The null hypothesis of equal effect of haplotypes within bull sire considering all sires simultaneously was rejected for protein yield ($P<.01$), protein percentage ($P<.10$) and milk yield ($P<.10$) but was accepted for fat yield. These results might indicate the presence of a QTL for milk protein production in the region encoding the four casein genes.

The contrasts between haplotype 5 and protein yield, protein percentage and milk yield analyzed within individual bull sires are given in Table 2. The actual increase in production associated to each haplotype is twice the values given in Table 2 since the contrasts are obtained from an analysis using a granddaughter design.

Table 2. Contrasts between casein haplotype 5 and protein yield, protein percentage and milk yield analyzed within individual bull sires. Values between brackets are standard errors.

Bull sire no.	Contrasts between haplotypes	Protein yield (kg)	Protein %	Milk yield (kg)
1	5 - 1	7.29 (2.16) **	.0164 (.0150)	197.4 (71.6) *
2	5 - 10	5.62 (2.37) †	.0237 (.0165)	122.8 (78.6)
3	5 - 4	3.12 (3.11)	.0127 (.0216)	65.6 (103.0)
4	5 - 3	1.26 (2.62)	.0347 (.0183)	- 34.6 (86.7)
5	5 - 1	1.93 (3.03)	.0322 (.0212)	- 17.2 (100.5)

†$P<.10$, *$P<.05$, **$P<.01$. Significance level for the contrasts was carried out using the Bonferroni Method.

A second analysis was performed with five bull sires containing haplotypes containing the B allele of κ-CN (haplotype 7, 8 and 9). No significant associations were found between these haplotypes and milk production traits.

Associations between casein alleles and milk protein production reported in the literature are summarized in Table 3. A positive association between the B allele of κ-CN and milk protein production traits is found in most, but not all, studies. The findings for α_{s1}-CN alleles are also inconsistent. One explanation of the various associations between casein alleles and milk production traits could be that there is not a direct effect of casein alleles on milk production traits. The correlation could rather be due to differences in regulatory regions of casein genes or variation in other loci linked to these genes. Differences in the linkage disequilibrium phase between the QTL and the alleles of casein loci across populations and breeds would then explain the findings reported in the literature and in different bull sire families in Norwegian Cattle.

Table 3. Genotypes of α_{s1}-CN and κ-CN positively associated with protein yield and protein percentage in a literature review.

LOCUS	TRAIT	Aleandri (1990)	Bovenhuis (1992)	Graml (1985)	Graml (1986)	Gonyon (1987)	Haenlein (1987)	Mao (1992)	Ng-Kwai-Hang (1984)
α_{s1}-CN	Prot. kg	BB *	BC	BB	CC	-	-	BC	BB **
	Prot. %	BC *	BC	BC *	CC	-	CC *	BC	BC
κ-CN	Prot. kg	BB †	BB	BB	AA	-	-	BB **	BB *
	Prot. %	BB **	BB ***	AA	AB	BB *	-	BB **	BB *

†$P \leq .10$, *$P \leq .05$, **$P \leq .01$, ***$P \leq .001$

Aleandri et al. (1990) n = 1383 Holstein Friesian, Bovenhuis et al. (1992) n = 6803 Holstein Friesian, Graml et al. (1985) n = 4241 Fleckviehs/Braunviehs, Graml et al. (1986) n = 4401 Fleckviehs/Braunviehs, Gonyon et al. (1987) n = 3111 Holstein Friesian, Haenlein et al. (1987) n = 3888 Guernseys, Mao et al. (1992) n = 4421 Holstein Friesian, Ng-Kwai-Hang et al. (1984) n = 2045 Holstein Friesian. n = the number of cows in each study.

THE USE OF CASEIN HAPLOTYPES IN THE IMPROVEMENT OF NORWEGIAN CATTLE

Despite the worldwide interest in associating genetic markers with quantitative trait loci, no markers have yet been utilized in genetic improvement of dairy cattle. The associations between haplotypes and milk production traits in Norwegian Cattle suggest the use of haplotype information in genetic improvement programs. The use of marker information in breeding programs can be accomplished by either selection across families or selection within families. The most efficient method might be to practice both in two stages. In the first stage, candidate bulls for progeny testing can be selected on the marker information and in the second stage on both the marker and the progeny test results. However, combining genotype and progeny information would require the assumption that the association of a particular allele with production traits is the same for all sires with that allele. Analysis was carried out within bull sire families and the observed associations could be the result of several QTL with different linkage phases within bull sire for the same or different haplotypes. If the linkage disequilibrium is loose then the efficiency of selection across families can be reduced. Furthermore, a preliminary study undertaken in our laboratory showed a negative association of haplotype 5 with mastitis and ketosis.

Alternatively, selection may be carried out within sire families using the information obtained from the contrast within sire families. This strategy has the additional advantage that the rate of inbreeding is not compromised. Research is ongoing to assess the economic value of within family selection for casein haplotypes in Norwegian Cattle.

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