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Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition

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ABSTRACT

The 6 main milk proteins in cattle are encoded by highly polymorphic genes characterized by several nonsynonymous and synonymous mutations, with up to 47 protein variants identified. Such an extensive variation was used for linkage analysis with the description of the casein cluster more than 30 yr ago and has been applied to animal breeding for several years. Casein haplotype effects on productive traits have been investigated considering information on the whole casein complex. Moreover, mutations within the noncoding sequences have been shown to affect the specific protein expression and, as a consequence, milk composition and cheesemaking. Milk protein variants are also a useful tool for breed characterization, diversity, and phylogenetic studies. In addition, they are involved in various aspects of human nutrition. First, the occurrence of alleles associated with a reduced content of different caseins might be exploited for the production of milk with particular nutritional qualities; that is, hypoallergenic milk. On the other hand, the frequency of these alleles can be decreased by selection of sires using simple DNA tests, thereby increasing the casein content in milk used for cheesemaking. Furthermore, the biological activity of peptides released from milk protein digestion can be affected by amino acid exchanges or deletions resulting from gene mutations. Finally, the gene-culture coevolution between cattle milk protein genes and human lactase genes, which has been recently highlighted, is impressive proof of the nonrandom occurrence of milk protein genetic variation over the centuries.
Key words: milk protein, cattle, genetic polymorphism

INTRODUCTION

More than 95% of the proteins contained in ruminant milk are coded by 6 structural genes (Martin et al., 2002; Figure 1). The 4 casein genes are tightly linked in a 250-kb cluster (Ferretti et al., 1990; Threadgill and Womack, 1990) mapped on chromosome 6 (Hayes et al., 1993; Popescu et al., 1996). In physical order, these genes are *CSN1S1*, *CSN2*, *CSN1S2*, and *CSN3* and encode α_{s1}-CN, β-CN, α_{s2}-CN, and κ-CN, respectively. This gene cluster is also referred to as the CN locus (Martin et al., 2002) or super locus (Freyer et al., 1999). The 2 main whey proteins, α-LA and β-LG, are coded by *LAA* and *LGB* genes, mapped on chromosomes 5 (Hayes et al., 1993) and 11 (Hayes and Petit, 1993), respectively.

Among ruminants, milk protein genes have been thoroughly investigated in cattle and goats, and a noticeable genetic variation has been identified and characterized. The importance of such an extensive genetic variation for animal breeding is mainly the consequence of the effects of milk protein variants on milk composition and cheesemaking properties (reviewed by Grosclaude, 1988; Di Stasio and Mariani, 2000; Martin et al., 2002). These effects are related to functional modifications of the protein, mainly AA exchanges or deletions, which affect the biological properties of the coded protein. In addition, milk protein variants were used for breed characterization (Moazami-Goudarzi et al., 2001; Cerioti et al., 2004a), biodiversity investigations (Lien et al., 1999; Mahé et al., 1999), and evolution studies on both animal resources and milk protein genes (Jann et al., 2004; Ibeagha-Awemu et al., 2007). For such studies, an interesting approach is to consider the whole CN haplotype instead of individual genes coding for the 4 caseins (Beja-Pereira et al., 2002).

In this review, we will concentrate our attention on the bovine species, aiming both to emphasize the pioneer work and exhaustive reviews that have already been carried out on the subject in the past 30 yr and to elucidate some of the main aspects affecting animal

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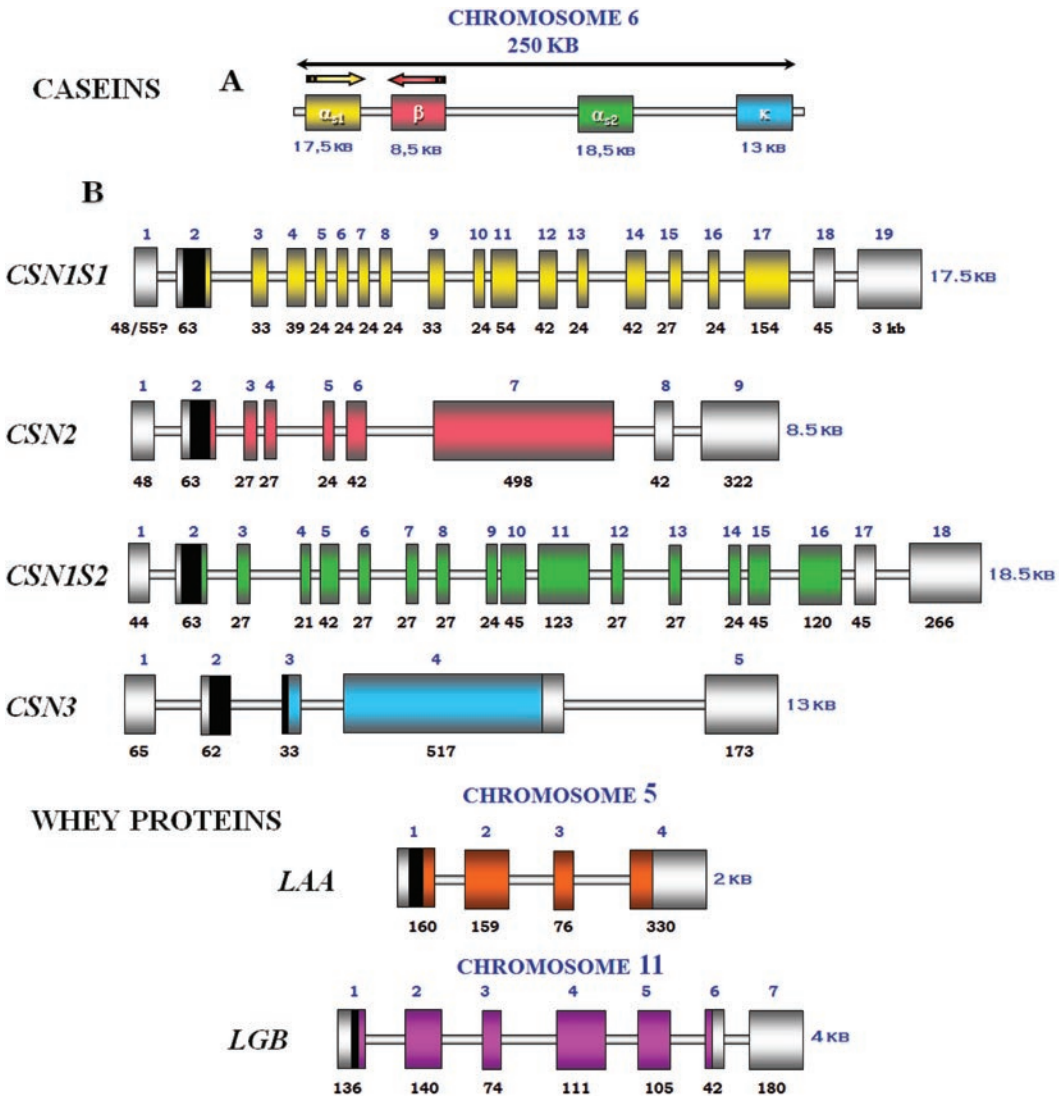

Figure 1. Structural organization of the transcription units encoding the 6 main milk proteins. Caseins: α_{s1}-CN (*CSN1S1*), β-CN (*CSN2*), α_{s2}-CN (*CSN1S2*), and κ-CN (*CSN3*). Whey proteins: α-LA (*LAA*) and β-LG (*LGB*). A) Genomic organization of the bovine casein locus. B) Structural organization of the 6 milk protein transcription units. Open bars represent introns; exons are depicted by large, gray (5′ and 3′ untranslated regions), black (part of exon encoding the signal peptide), and colored (exons and part of exons encoding matured proteins) boxes. Size of exons is given, in base pairs, under each exon with its number indicated on the top (modified from Martin et al., 2002).

breeding and human nutrition, with special focus on innovative scientific approaches.

BOVINE MILK PROTEIN VARIANTS

Studies on milk protein genetic variability started more than 50 yr ago by detecting bovine β-LG main variants (Aschaffenburg and Drewry, 1957) and intensified during the following years, discovering polymorphisms with important differences among bovine species and breeds (reviewed by Formaggioni et al., 1999). Genetic variants can result from SNP, as well as nucleotide (**nt**) deletions or insertions. A recent review of milk protein nomenclature (Farrell et al., 2004) indicated 8 α_{s1}-CN (*A*, *B*, *C*, *D*, *E*, *F*, *G*, *H*), 4 α_{s2}-CN (*A*, *B*, *C*, *D*), 12 β-CN (A^1, A^2, A^3, *B*, *C*, *D*, *E*, *F*, *G*, H^1, H^2, *I*), 11 κ-CN (*A*, *B*, *C*, *E*, F^1, F^2, G^1, G^2, *H*, *I*, *J*), 11 β-LG (*A*, *B*, *C*, *D*, *E*, *F*, *G*, *H*, *I*, *J*, *W*), and 3 α-LA (*A*, *B*, *C*) variants in the *Bos* genus. The nomenclature is unified for the 4 species of *Bos* genus mainly considered in cattle milk protein studies; that is, *Bos taurus* (taurine bovine), *Bos indicus* (zebu), *Bos grunniens* (yak), and *Bos javanicus* (banteng of Bali). Only protein polymorphisms are considered in this review, namely mutations

modifying the AA sequence of the coded protein, with the exception of *CSN1S1*G* differing from *CSN1S1*B* for a lower protein expression (Rando et al., 1998).

In addition to the review by Farrell et al. (2004), a novel α_{s1}-CN protein variant, named *CSN1S1*I*, was recently characterized (Lühken et al., 2009). An A > T SNP substitution in exon 11 leads to the substitution Glu > Asp at AA position 84 of the mature protein. Lühken et al. (2009) postulated that *CSN1S1*I* originated within *Bos indicus* and spread to *Bos taurus* subsequently. Thus, the number of α_{s1}-CN protein variants increases to 9. The characterization of α_{s2}-CN**B* has also been performed, and a C > T exchange has been identified in the 17th nt of exon 3, leading to the Ser > Phe substitution in the eighth amino acid of the mature protein (Ibeagha-Awemu et al., 2007).

As for *CSN3*, the B^2 variant characterized by Gorodetskiĭ and Kaledin (1987) can be added to the list of Farrell et al. (2004). This variant is available on Swiss-Prot (no. P02668). More details on *CSN3*C* and *CSN3*G*^{2}, as per nomenclature of Farrell et al. (2004), are given in the following.

Conflicting data have been reported about *CSN3*C*, which was first described in the Grey Alpine (Di Stasio and Merlin, 1979) as a variant migrating faster than *CSN3*A* by alkaline electrophoresis. Mariani (1983) found a similar migrating variant in the Italian Brown and suggested it was the same as that in the Grey Alpine. However, no characterization of the variant was carried out in either breed. Later, Seibert et al. (1987) named *CSN3*D*, a variant migrating in the same position as *CSN3*A* by alkaline electrophoresis but showing an intermediate position between *CSN3*A* and *CSN3*B* by isoelectrofocusing (**IEF**). The correspondence of such a variant with *CSN3*C* was shown by IEF (Krause et al., 1988); thus, *CSN3*D* disappeared from cattle milk protein nomenclature, not taking into account the different migration observed by alkaline electrophoresis. Miranda et al. (1993), following the new nomenclature, characterized *CSN3*C*, namely the old *CSN3*D*, as a counterpart of *CSN3*B*. An unassigned *CSN3* sequence DNA (GenBank No. AJ619772) demonstrates the existence of a variant carrying His_{97}, as *CSN3*C* does, that is identical to *CSN3*A* at the other positions. The variant was found in Italian Brown cattle and migrated by alkaline gel faster than *CSN3*A* but at an intermediate position between *A* and *B* by IEF (A. M. Caroli; unpublished results). These data clearly indicate the existence of 2 variants carrying His_{97}, which are the counterparts of *CSN3*B* and *CSN3*A*, respectively. We would propose to maintain the name *CSN3*C* for the former and to give the latter the name *CSN3*D*, which is actually missing in the *Bos* genus nomenclature.

The *CSN3*G*^{2} variant, first described in the yak by Sulimova et al. (1996), was recently confirmed by Prinzenberg et al. (2008) in the same species in which at least 2 yak-specific DNA sequences were found. All yak had nt sequences coding for Thr in AA position 136 (identical to bovine *CSN3*A*) and Ala in position 148 (identical to bovine *CSN3*B*). A 12-bp insertion in the coding region, representing a repeated nt and AA motif, was found in one allele, as well as differences in the stop codon sequences. The loss of the insertion might have led to the ancestral *CSN3* allele from which all currently known variants of *CSN3* in the *Bos* genus evolved. In *Bos taurus,* a further *CSN3* allele was found that coded for Thr_{136} and Ala_{148} (Chessa et al., 2007). This variant, which can be considered to be the wild type for the *Bos* genus, was tentatively named *CSN3*W*. The exact correspondence with *CSN3*G*^{2} should be verified for the synonymous variations identified in the yak. In addition, Chen et al. (2008) found a *CSN3* intragenic haplotype in the gayal (*Bos frontalis*) and zebu, differing from *CSN3*B* for the occurrence of Thr_{136} instead of Ile_{136} and showing a close phylogenetic relationship with the banteng, gaur, and yak. In the latter, a G > C SNP substitution resulting in an AA substitution from Arg_{121} to Pro_{121} of the mature protein was also found (Bai et al., 2008). These data highlight the possible need to introduce separate nomenclature for milk protein variants, which can differ greatly among species within the *Bos* genus.

GENOTYPING SYSTEMS

Genetic variation can be detected at the phenotypic level by different protein identification techniques (i.e., electrophoresis, IEF, chromatography). The electrophoretic and isoelectrophoretic techniques, mainly used for routine typing at the protein level, only allow detection of genetic variation resulting in different electrophoretic or isoelectrophoretic mobility, such as AA exchanges altering the electric charge or the isoelectric point of the protein. For the screening of breeds and populations at the phenotypic level, IEF is still the most effective method and should be recommended for typing animals reared on continents where breed characterization using milk protein genes is still limited (e.g., Africa and Asia). For biodiversity studies, and if milk is available, typing at the protein level by IEF is recommended instead of analyzing just one milk protein at the DNA level because the method is cheap and fast and gives a simultaneous picture of the phenotype expression of the 6 main milk protein genes (Erhardt and Eggen, 1990). After this phenotypic screening, more detailed studies might be carried out at the DNA level for a wider scan

Table 1. Position within the gene and mature protein of the *CSN1S1* genetic variants in *Bos* genus[1]

	CSN1S1 variant								
Gene, Protein	*B*	*A*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
14891–14929									
14–26		Del.[2]							
17383	**G**CC			**A**CC					
53	Ala			ThrP					
17377–17400									
51–58								Del.	
18901	**C**AA				**A**AA				
59	Gln				Lys				
18923	T**C**G					T**T**G			
66	SerP					Leu			
19836	GA**A**								GA**T**
84	Glu								Asp
26181	G**A**A		G**G**A		G**G**A				
192	Glu		Gly		Gly				

[1]The reference sequence is GenBank No. X59856, corresponding to the *CSN1S1*B* allele. Nonsynonymous mutations are bolded within each codon. *CSN1S1*E* was found only in *Bos grunniens*.

[2]Del. = Deleted.

of the chromosome regions coding for the respective milk proteins.

Certainly, DNA analysis has given a new impulse to investigations of bovine milk protein polymorphisms, allowing the identification of known protein variants at the genome level by different techniques, such as PCR-RFLP for *CSN3* (Damiani et al., 1990) and *LGB* typing (Medrano and Aguilar-Cordova, 1990), direct sequencing for *CSN3* (Schlieben et al., 1991), allele-specific-PCR for *CSN2* (Damiani et al., 1992) and *CSN1S1* (David and Deutch, 1992), and PCR-single-strand conformation polymorphism for *CSN2* (Barroso et al., 1999) and *CSN1S1* typing (Jann et al., 2002a). In addition, further nt mutations were identified at the DNA level that either modify the protein sequence (i.e., *CSN2*I*; Jann et al., 2002b), or result in synonymous protein variants because the nt substitution within the codon does not modify the correspondent AA. This is the case with *CSN3*A1* (Damiani et al., 1990) and *CSN3*A*^{I} (Prinzenberg et al., 1999), both sharing the protein sequence with *CSN3*A*, as well as *CSN2*A*$^{2\prime}$(Ceriotti et al., 2004a), which codes for the same protein as *CSN2*A*^{2}. Tables 1, 2, 3, 4, 5, and 6 show the main milk protein variants that have been identified, using the nomenclature proposed by Farrell et al. (2004) and including the synonymous variant *CSN3*A*^{I} because of its presence in many *Bos indicus* breeds (Jann et al., 2004). Table 7 combines the available information on the distribution and discovery of the genetic variants reported. Although the number of genetic variants identified and characterized in the *Bos* genus is very large, only a few variants (12 of 53) are widely distributed in *Bos taurus* and *Bos indicus*, 34 are less common or rare in these 2 species, and 7 occur only in *Bos grunniens* and/or *Bos javanicus*. This can set the priority of typing particular alleles based on the species and breed of interest.

Typing at the DNA level does not require the gene product, which renders feasible the genotyping of males and nonlactating females. Nevertheless, DNA extracted from milk somatic cells can also be used for the molecular analyses (Chessa et al., 2007), taking advantage of easier sampling of cows recorded for milk production.

Milk protein polymorphisms can provide useful information for identity control within official milk recording systems by analyzing individual milk by IEF (Erhardt and Senft, 1991). They can also be used as genetic markers for parentage testing. For the latter, application of high-throughput assays such as the microarray technology (Chessa et al., 2007) can lead to wide-scale animal genotyping at several SNP within the milk protein genes. In addition, the possibility of using particular milk protein variants to trace the origin of typical products derived from local breeds could be further investigated in light of encouraging results already obtained in goats (Ceriotti et al., 2004b).

MILK PROTEIN VARIANTS AND CHEESEMAKING PROPERTIES

One of the most striking effects of the milk protein polymorphisms on traits with economic interest is their relation with cheesemaking properties of milk; this effect has been mainly investigated in cattle (reviewed by Di Stasio and Mariani, 2000). Studies were conducted in Italy in the 1970s (i.e., Losi et al., 1973; Mariani et al., 1976), many of them focusing on the effects of κ-CN on the rheological quality of milk. The κ-CN, located predominantly on the casein micelle surface, is the specific substrate of the chymosin, the hydrolytic

Table 2. Position within the gene and mature protein of the *CSN2* genetic variants in *Bos* genus[1]

	CSN2 variant[2]											
Gene, Protein	*A*[1]	*A*[2]	*A*[3]	*B*	*C*	*D*	*E*	*F*	*G*	*H*[1]	*H*[2]	*I*
4647–4648	A**G**C					?[3]						
18	SerP					Lys						
6562	**C**GC									**T**GC		
25	Arg									Cys		
6684–6686	AGT				AGT							
35	SerP				Ser							
6687	**G**AG						**A**AG					
36	Glu						Lys					
6690	**G**AA				**A**AA							
37	Glu				Lys							
8101	C**A**T	C**C**T	C**C**T			C**C**T	C**C**T			?	C**C**T	C**C**T
67	His	Pro	Pro			Pro	Pro			?	Pro	Pro
8115	**C**AA										**G**AA	
72	Gln										Glu	
8163	**C**TT									**A**TT		
88	Leu									Ile		
8178	**A**TG										**C**TG	**C**TG
93	Met										Leu	Leu
8219	CA**C**		CA**A**									
106	His		Gln									
8267	AG**C**			AG**G**								
122	Ser			Arg								
8311–8314[4]	[4]								[4]			
137–138[4]	Pro								Leu			
8356	C**C**T							C**T**T				
152	Pro							Leu				
??[5]	??										??	
??	Gln										Glu	

[1]The reference sequence is GenBank No. X14711, corresponding to the *CSN2*A*[1] allele. Nonsynonymous mutations are bolded within each codon. *CSN2*D* was found only in *Bos indicus*.

[2]*H*[1] = H of Han et al. (2000); *H*[2] = H of Senocq et al. (2002).

[3]? = Information not available.

[4]At position 137–138, a Pro–Leu inversion is controversial depending on the sequences (Farrell et al., 2004).

[5]?? = Substitution of Gln to Glu is described between residues 114–169 (Senocq et al., 2002).

Table 3. Position within the gene and mature protein of the *CSN1S2* genetic variants in *Bos* genus[1]

	CSN1S2 variant			
Gene, Protein	*A*	*B*	*C*	*D*
6227	T**C**C	T**T**C		
8	Ser	Phe		
7568	G**A**G		G**G**G	
33	Glu		Gly	
8401	**G**CA		**A**CA	
47	Ala		Thr	
8853–8879				
51–59				Del.[2]
8879	GA**G**			GA**T**
59	Glu			(Asp)-[3]
11018	A**C**C		A**T**C	
130	Thr		Ile	

[1]The reference sequence is GenBank No. M94327, corresponding to the *CSN1S2*A* allele. Nonsynonymous mutations are bolded within each codon. *CSN1S2*C* was found only in *Bos grunniens*.

[2]Del. = Deleted.

[3]- = Deleted within the protein, but the gene sequence changed.

activity of which splits the κ-CN into the insoluble para-κ-CN (amino acid 1–105) and the soluble caseinomacropeptide (**CMP**: amino acid 106–171). This is a crucial process for the production of cheese but also for the nutrition of suckling calves (Mercier et al., 1973). Important physiological functions such as increasing digestion efficiency (Mercier et al., 1976) and antibacterial activity (Malkoski et al., 2001) were ascribed in particular to CMP.

It is well known that milk with the *CSN3*B* variant reacts more promptly with rennet and has a rennet coagulation time significantly shorter than milk with *CSN3*A*, whereas milk from heterozygous cows shows an intermediate behavior (Losi et al., 1973). Differences in the micelle stability that occur between the 2 genetic variants *CSN3*A* and *CSN3*B* are strictly connected to the micelle size and the glycosylation degree of the protein itself (Di Stasio and Mariani, 2000). Nevertheless, less-common *CSN3* alleles might affect milk rheological properties, too. The constant monitoring of milk protein variation in different breeds of cattle is an essential practice aiming to avoid an increase in frequencies of mutations with unfavorable effects on cheesemaking. An example of a rare allele with a negative effect on rheological traits is *CSN3*G*, identified in the Pinzgauer breed and associated with more unfavorable coagulation properties than *CSN3*A* (Erhardt et al., 1997). Similarly, in the Italian Friesian, a negative effect of the *CSN3*E* variant was detected on milk clotting traits (Caroli et al., 2000). Although κ-CN is a crucial element in renneting, interactions with the other milk protein systems have to be taken into account, in particular, β-CN and β-LG. In general, *CSN2*B* and *LGB*B* were found to be more favorable for rennet coagulation and the cheesemaking quality of milk (Di Stasio and Mariani, 2000). Composite CN genotypes (Aleandri et al., 1990; Comin et al., 2008) were also considered because of the tight genetic linkage among the CN genes. In Italian Holsteins, Comin et al. (2008) found *CSN3* and *CSN2* to be strongly associated with milk coagulation traits and milk and protein yields, respectively, and proposed the composite genotypes at both genes to be the most appropriate criterion for selection decisions. For coagulation time and curd firmness, the best *CSN2–CSN3* composite genotypes were those with at least one *B* allele at both loci.

Table 4. Position within the gene and mature protein of the *CSN3* genetic variants in *Bos* genus[1]

	CSN3 variant[2]													
Gene, Protein	*A*	A^1	*B*	B^2	*C*	*D*	*E*	F^1	F^2	G^1	G^2	*H*	*I*	*J*
12690	C**G**C								C**A**C					
10	Arg								His					
12940	AC*T*			AC*C*										
93	Thr			Thr										
12950	**C**GT									**T**GT				
97	Arg									Cys				
12951	C**G**T				C**A**T	C**A**T								
97	Arg				His	His								
12971	**T**CA												**G**CA	
104	Ser												Ala	
13065	A**C**C									A**T**C		A**T**C		
135	Thr									Ile		Ile		
13068	A**C**C		A**T**C	A**T**C	A**T**C									A**T**C
136	Thr		Ile	Ile	Ile									Ile
13096	AC*T*							AC*G*						
145	Thr													
13104	G**A**T		G**C**T	G**C**T	G**C**T			G**T**T			G**C**T			G**C**T
148	Asp		Ala	Ala	Ala			Val			Ala			Ala
13111	CC*A*	CC*G*												
150	Pro													
13119	A**T**T			A**C**T										
153	Ile			Thr										
13124	**A**GC						**G**GC							?[3]
155	Ser						Gly							Arg
13162	AC*T*			AC*C*							AC*C*			
167	Thr													
13165	GC*A*		GC*G*	GC*G*	GC*G*	?					GC*G*			
168	Ala													

[1]The reference sequence is GenBank No. AY380228, corresponding to the *CSN3*A* allele. Nonsynonymous and synonymous mutations are respectively bolded and in italics within each codon.

[2]F^1 = F of Sulimova et al. (1992); F^2 = F of Prinzenberg et al. (1996), GenBank no. AF123250; G^1 = *G* of Erhardt (1996), Prinzenberg et al. (1996), GenBank no. AF123251; G^2 = *G* of Sulimova et al. (1996); *D* = GenBank No. AJ619772.

[3]? = Information not available.

MILK PROTEIN EVOLUTION

Interspecies comparisons of cDNA and milk protein genes have confirmed their high rate of evolution, but the overall gene organization has been conserved (Mercier and Vilotte, 1993). The CN genes appear to be a rapidly evolving family, presumably because of minimal structural requirements for function (Bonsing and Mackinlay, 1987). Sequence alignments of CN genes from various species reveal evidence of a high mutation rate including insertions, deletions, and sequence rearrangements (Bawden and Nicholas, 1999). Nevertheless, comparative analysis of CN genomic sequences of human, rodent, and cattle shows that the organization and orientation of the genes is greatly conserved, and the molecular diversity of the CN genes is achieved through variable use of exons in different species and great evolutionary divergence (Rijnkels, 2002). Nutritionally, caseins provide the suckling neonate with a source of amino acids, highly bioavailable calcium, and potential bioactive peptides (i.e., antimicrobial, immune modulating).

Interspecies comparisons demonstrated that *CSN3* possesses the greatest degree of conservation among the CN genes, which might be related to its essential function of CN micelle stabilizer (Alexander et al., 1988). Investigation at the DNA level allows the comparison between synonymous and nonsynonymous mutations. Gatesy et al. (1996) compared *CSN3* exon IV of 21 mammalian taxa and reported similar rates for synonymous and nonsynonymous substitutions per site, with the divergence at each codon position being roughly equivalent. This pattern of divergence at *CSN3* was interpreted by the authors as supporting a strictly neutral model of evolution for this gene. In contrast with a neutral evolution model, Ward et al. (1997) reported strong positive selection on the CMP between distantly related bovid taxa, leading to an accelerated divergence of this peptide within a 34-codon region. In closely related species, this pattern was not observed. Consequently, the authors predicted less polymorphism of CMP within species and more extensive polymorphism between species.

The availability of the *Bos taurus* genome sequence assembly marks the beginning of a new era for the study of milk and mammary biology. Using this assembly, Lemay et al. (2009) identified 197 unique milk protein genes and found the genes encoding milk components and other genes expressed in the mammary gland to be under more stringent selection constraints (negative selection) compared with the rest of the genome, aiming to maximize the survival of both mother and offspring. This highlights the importance of milk in mammalian evolution. Evidence of positive selection, even if not significantly different under the likelihood ratio test, was found for *CSN2* and *CSN3* (Lemay et al., 2009). The authors concluded that the requirement that the entire gene show statistical evidence of positive selection might be too stringent and further site-specific evolutionary analysis of the casein genes might be warranted. Thus, the debate on the evolution model of *CSN3* still needs further investigations in light of the more recent molecular knowledge.

Table 5. Position within the gene and mature protein of the *LAA* genetic variants in *Bos* genus[1]

	LAA variants		
Gene, Protein	*B*	*A*	*C*
851	C**G**G	C**A**G	
10	Arg	His	
?[2]	?		?
?	Asp?		Asn?

[1]The reference sequence is GenBank No. X06366, corresponding to *LAA*B*. *LAA*C* was found only in *Bos javanicus*.

[2]? = The *C* variant was reported to differ from *B* by having either an Asn-for-Asp or a Gln-for-Glu substitution (Bell et al., 1981).

This type of research is indeed very attractive because the main interest in milk protein polymorphism studies is to understand the biological significance of the genetic variation, which can be highlighted by evolutionary studies.

THE CN HAPLOTYPE AND SIRE SELECTION

As already mentioned, knowledge of CN gene variation at the haplotype level has been a useful tool in biodiversity studies. As an example, the comparison of African *Bos taurus* and *Bos indicus* breeds allowed the identification of several *Bos indicus*–specific haplotypes (*CSN1S1*C-CSN2*A*^{2}*-CSN3*A*^{I} / *CSN3*H*) that were not found in pure taurine breeds. The occurrence of such haplotypes in southern European breeds suggested that an introgression of indicine genes into taurine breeds could have contributed to the distribution of the genetic variation observed (Jann et al., 2004).

Casein haplotype effects on productive traits have also been investigated, considering information on the whole CN complex. The latter approach is recommended for both research and breeding purposes, possibly taking into account all CN variants, as well as other important polymorphisms in the noncoding regions. Instead of analyzing effects of single alleles, a series of studies has focused on the estimation of CN haplotype effects, as discussed in the following.

Lien et al. (1995) considered other sites of polymorphism within the CN area of chromosome 6 and found a significant favorable effect of a paternal haplotype on

Table 6. Position within the gene and mature protein of the *LGB* genetic variants in *Bos* genus[1]

	LGB variant										
Gene, Protein	*B*	*A*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*W*
3065	**G**AG			**C**AG[2]							
45	Glu			Gln							
3080	**C**CT					**T**CT[2]					
50	Pro					Ser					
3098	**A**TC										**C**TC[2]
56	Ile										Leu
3109	CA**G**		CA**T**								
59	Gln		His								
3982	AA*C*	AA*T*			?[3]	?	?	?	?	?	?
63	Asn										
3984	G**G**T	G**A**T						G**A**T			
64	Gly	Asp						Asp			
4003	AA**G**							AA**?**			
70	Lys							Asn			
4027	AT**C**						AT**G**				
78	Ile						Met				
5174	AA*T*	AA*C*	?	?	?	?	?	?	?	?	?
88	Asn										
5233	G**A**G								G**G**G[2]		
108	Glu								Gly		
5263	G**C**C	G**T**C						G**T**C[2]			
118	Ala	Val						Val			
5962	C**C**G									C**T**G[2]	
126	Pro									Leu	
5970	**G**AC					**T**AC[2]					
129	Asp					Tyr					
6280	G**A**G				G**G**G[2]	G**G**G[2]	G**G**G[2]				
158	Glu				Gly	Gly	Gly				

[1]The reference variant is *LGB*B* (GenBank No. X14710). Nonsynonymous and synonymous mutations are respectively bolded and in italics within each codon. *LGB*E* was found in *Bos grunniens* and *Bos javanicus*, *LGB*F* and *LGB*G* only in *Bos javanicus*.

[2]The most probable codon is reported, considering that only one SNP occurred within the triplet.

[3]? = Information not available.

milk protein yield in a Norwegian cattle family. This haplotype was named *C-A5–16-A* because it carries, in addition to *CSN1S1*C*, the *CSN2*A*^{5} allele, which shows a silent C > T mutation in codon 110, the mature protein not being different from *CSN2*A*^{2} (Lien and Rogne, 1993). This haplotype also carries a microsatellite in *CSN3* intron 3 with 16 repeats instead of 14, as well as allele *CSN3*A*.

The analysis of the effects of paternal haplotypes within sires revealed the opposite effect of haplotypes for 2 of the 7 sires for casein content (Braunschweig et al., 2000). This inconsistency gave rise to speculation that the κ-CN allele might be connected to different promoters or cis-acting regulatory sequences. Various significant effects have been observed by Boettcher et al. (2004), particularly for protein content in milk, in Italian Holstein and Brown breeds, in agreement with previous scans of chromosome 6 (Kühn et al., 1999; Velmala et al., 1999). Among the haplotypes, *B-A*^{1}*-B* (in the order: *CSN1S1-CSN2-CSN3*) was associated with increased percentage of both fat and protein in Finnish Ayrshire (Ikonen et al., 2001) and Italian Holstein and Brown cattle but had negative effects on milk yield (Boettcher et al., 2004). The *C-A*^{2}*-B* haplotype was associated with significantly decreased yield and increased concentration of protein, whereas, in general, haplotypes carrying the *CSN3*B* allele had positive effects on protein percentage relative to the corresponding haplotypes carrying *CSN3*A* (Boettcher et al., 2004). In a recent investigation of the Dutch Holstein-Friesian population, Heck et al. (2009) concluded that selection for *CSN2-CSN3* haplotype *A*^{2}*-B*, together with *LGB*B*, would result in cows that produce milk more suitable for cheesemaking.

The main problems in comparing research studies considering haplotypes are i) the different points of mutations used for constructing the casein haplotype and ii) difficulties in reconstructing the exact haplotypes carried by each animal. Besides the genotype of the animals at the single polymorphism of interest, both pedigree information and parent typing at the same polymorphisms should be available for an accurate diagnosis, mainly if several mutations are considered within the CN region. In all cases, correlations among

Table 7. Distribution and discovery year of the milk protein variants reported in Tables 1–6[1]

Allele[2]		Diffusion	Species[3]	Breeds[4]	Year
CSN1S1	*A*	Rather common	T	Holstein-Friesian, Red Danish, German Red, Kostroma, other Friesian	1962
	B	Most common	T, I, G	All breeds	1962
	C	Common	All	All breeds; very common in *Bos indicus* breeds	1962
	D	Rather common	T	Flamande, Red Danish, Red Polish, Jersey, Italian Brown, German Holstein, etc.	1966
	E	Common	G		1974
	F	Rare	T	German Black and White, Italian Brown, Ayrshire, etc.	1992
	G	Rather common	T	Italian Brown, Podolian, other Italian breeds, Carora	1992
	H	Rare	T	Kuri	1992
	I	Rather common	T, I	Carora, Turkish Red Steppe, Banyo Gudali, etc.	2002
CSN2	A^1	Common	T, I	Most breeds	1966
	A^2	Most common	All	All breeds	1966
	A^3	Rather common	T, I	Holstein-Friesian, Jersey, Simmental, Sahiwal, German Red Pied, etc.	1966
	B	Common	T, I	Most *taurus* breeds, Hariana, Choa	1961
	B^2	Rare	T	Russian cattle	1986
	C	Rather common	T	Guernsey, Reggiana, Pinzgauer, Italian Brown, Piemontese, etc.	1961
	D	Rare	I	Indian Deshi, East African Boran	1968
	E	Rare	T	Piemontese	1972
	F	Rare	T	Meuse-Rhine-Yssel	1991
	G	Rare	T	Holstein-Friesian	1997
	H^1	Rare	T	Korean cattle	1983
	H^2	Rare	T	Normande	2002
	I	Rather common	T	Italian Red Pied, Italian Holstein, German Holstein, Belgian Blue, Jersey, etc.	2002
CSN1S2	*A*	Most common	All	All breeds	1976
	B	Common	I, T, G	All *Bos indicus* breeds, Turkish Gray Steppe, Namchi, Anatolian Black, etc.	1976
	C	Rare	G	Mongolia breeds	1976
	D	Rather common	T	Montbéliarde, Vosgienne, German Yellow and Simmental, Menorquina, Ayrshire	1976
CSN3	*A*	Most common	T, I	All breeds	1964
	A^1	Rather common[5]	I, T	Boran, Brahman, Nelore, Santa Gertrudis, etc.	1999
	B	Most common	I, T	All breeds	1964
	C	Rather common	T	Grey Alpine, Italian Brown, German Simmental, Menorquina, etc.	1978
	D	Rare[6]	T	Italian Brown	1978
	E	Rather common	T	Angler, German Black and White, Holstein-Friesian, Ayrshire	1989
	F^1	Rare	T	Yakut	1992
	F^2	Rare	T	Finnish Ayrshire	1996
	G^1	Rare	T	Pinzgauer	1996
	G^2	Common[7]	G		1996
	H	Common[8]	I, T	Madagascar zebu, White Fulani, Wadara, Red Bororo, N'Dama, Pinzgauer	1974
	I	Rare	T × I	Namibia	1998
	J	Rare	T	Ivory Coast, Burkina Faso	1999
LAA	*A*	Rather common	I, T	All *indicus* and some *taurus* breeds	1958
	B	Most common	T, I, G	All breeds	1958
	C	Rare	J	Australia breeds	1981
LGB	*A*	Most common	T, I, G	All breeds	1955
	B	Most common	T, I, G	All breeds	1955
	C	Rather common	T	Jersey	1962
	D	Rather common	T	Montbéliarde, German Holstein, German Simmental, etc.	1966
	E	Most common	G, J	Nepal *grunniens*, Australia *javanicus*	1976
	F	Rare	J		1981
	G	Rare	J		1981
	H	Rare	T	Italian Friesian	1987
	I	Rare	T	Polish Red	1998
	J	Rare	T	Hungarian Grey	1993
	W	Rare	T	Murnau-Werdenfelser, Jersey, Red Holstein × Simmental	1980

[1]Information is taken from the survey by Formaggioni et al. (1999) and from Boettcher et al. (2004), Jann et al. (2004), Ibeagha-Awemu et al. (2007), and Caroli et al. (2008).

[2]Superscripts indicate different variants.

[3]T = *Bos taurus*; I = *Bos indicus*; G = *Bos grunniens*; J = *Bos javanicus.*

[4]When a list of breeds is reported, the first breed is the one in which the allele was first identified.

[5]Rather high frequencies in some *Bos indicus* breeds; also found in Boran × N'Dama, Friesian × Sahiwal crosses.

[6]Depending on the exact molecular characterization of the allele, it could be more common (see text).

[7]Other variants were recently identified in *Bos grunniens* (see text), and more details about their distribution are needed.

[8]Mainly in *Bos indicus*.

breeds tended to be high and positive, indicating that haplotypes had similar effects in the different breeds and suggesting that the CN genes themselves were responsible for the haplotype effects observed, rather than genes physically linked to the CN complex (Boettcher et al., 2004).

Nilsen et al. (2009) recently constructed a high-resolution SNP map of the bovine CN region to study associations with milk traits in Norwegian Red cattle and suggested separation of the CN cluster into 2 haplotype blocks, one consisting of *CSN1S1*, *CSN2*, and *CSN1S2*, and the other of *CSN3*. Highly significant associations with both protein and milk yield were found within the *CSN1S1-CSN2-CSN1S2* haplotype block. In contrast, no significant association was found within the *CSN3* block. The authors pointed toward *CSN2* and *CSN1S2* as the most likely genes harboring the underlying causative DNA variation. The most significant results involved the *CSN2_67* SNP, a C > A nt substitution in codon 67 that results in the AA exchange Pro to His, with C being consistently associated with greater protein and milk yield. Of the 12 *CSN2* variants (Table 2), A nt occurs in *CSN2*A*^{1}, *CSN2*B*, *CSN2*C*, and the less common *CSN2*F* and *CSN2*G* within a triplet coding for His_{67} in the mature protein, whereas C nt occurs in *CSN2*A*^{2}, *CSN2*A*^{3}, and in the other *CSN2* variants, resulting in Pro_{67}.

This short overview of the CN cluster confirms the importance of taking the whole haplotype into account for breeding strategies. The effects of alleles or SNP within the CN cluster determined by different authors in different breeds are sometimes conflicting (Nilsen et al., 2009), similar to QTL scans of chromosome 6 (Olsen et al., 2005). An explanation of such inconsistent findings might be that the haplotype balances, in some still unknown way, the constraints imposed by natural and/or artificial selection at the DNA region harboring the 4 CN genes, which are essential for both newborn cattle survival and breeding purposes. Recently, several QTL affecting milk protein composition (casein, whey protein, and specific protein content) were found, with the most significant regions being on *Bos taurus* autosomes 6, 11, and 14 (Schopen et al., 2009). The future use of genomic selection in animal breeding will have to take into account not only single SNP information but also particular "hot" zones of the animal genome, such as the CN cluster, where interactions among coding and noncoding nt can strongly influence the overall gene expression.

NONCODING POLYMORPHISMS

The so-called noncoding DNA variants are located in the noncoding regions of genes (5′-untranslated region including promoters, 3′-untranslated region, and introns or intragenic regions), as well as intergenic regions, and some of them have significant associations with production traits (Ibeagha-Awemu et al., 2008).

The noncoding sequences of milk protein genes have been intensively investigated. Important mutations altering the specific protein expression, and therefore milk composition, were found. Intronic mutations might affect splice sites and, consequently, mRNA stability and lead to truncated protein products or even lack of them. In bovine *CSN1S1*A*, an SNP at position +6 in the splice donor sequence of exon 4 results in upstream exon skipping during pre-mRNA processing, with reduced α_{s1}-CN expression (Mohr et al., 1994).

The bovine *CSN1S1*G* allele, associated with lower proportion of α_{s1}-CN in milk, is characterized by a 371 insertion in noncoding exon 19 (Rando et al., 1998). The smaller amount of α_{s1}-CN associated with *CSN1S1*G* can be explained by a reduced mRNA stability resulting from the insertion, which has a high homology with relics of long interspersed elements of retropositional origin. An SNP within a short interspersed nt element Bov-A2 was described by Damiani et al. (2000b) in the second intron of *CSN3* and found to be in linkage disequilibrium with the κ-CN protein variants (Damiani et al., 2000a) and also associated with several milk production traits (Damiani et al., 2001).

Polymorphisms of the *CSN1S1* promoter have revealed significant associations with milk protein content (Prinzenberg et al., 2003). Thus, the CN cluster polymorphism has to be considered as a whole complex in which expression sequence polymorphisms could help explain the productive implications of the different CN loci and their corresponding haplotypes.

Examples of noncoding variants are also known to affect whey proteins. In numerous studies, starting from Cerbulis and Farrell (1975), a greater protein expression level of the β-LG A variant compared with the B variant has been reported. Two SNP lead to AA changes and are the causal genetic polymorphisms of the protein variants β-LG A and β-LG B respectively coded by *LGB*A* and *LGB*B*. The functional reason for differences in expression between the 2 alleles has been investigated by several authors. Wagner et al. (1994) described 14 SNP in the 5′-flanking region and 2 SNP in the 5′-untranslated region of *LGB*. Lum et al. (1997) found 10 polymorphic sites distinguishing *LGB*A* and *LGB*B* promoters, one of which, a G > C transversion at position −430, is located within a consensus binding site for activator protein-2. The differential affinity of activator protein-2 to *LGB*A* and *LGB*B* promoters has been demonstrated in vitro, being 60% greater for the activator protein-2 recognition site of *LGB*A* (Lum et al., 1997). Folch et al. (1999) confirmed the

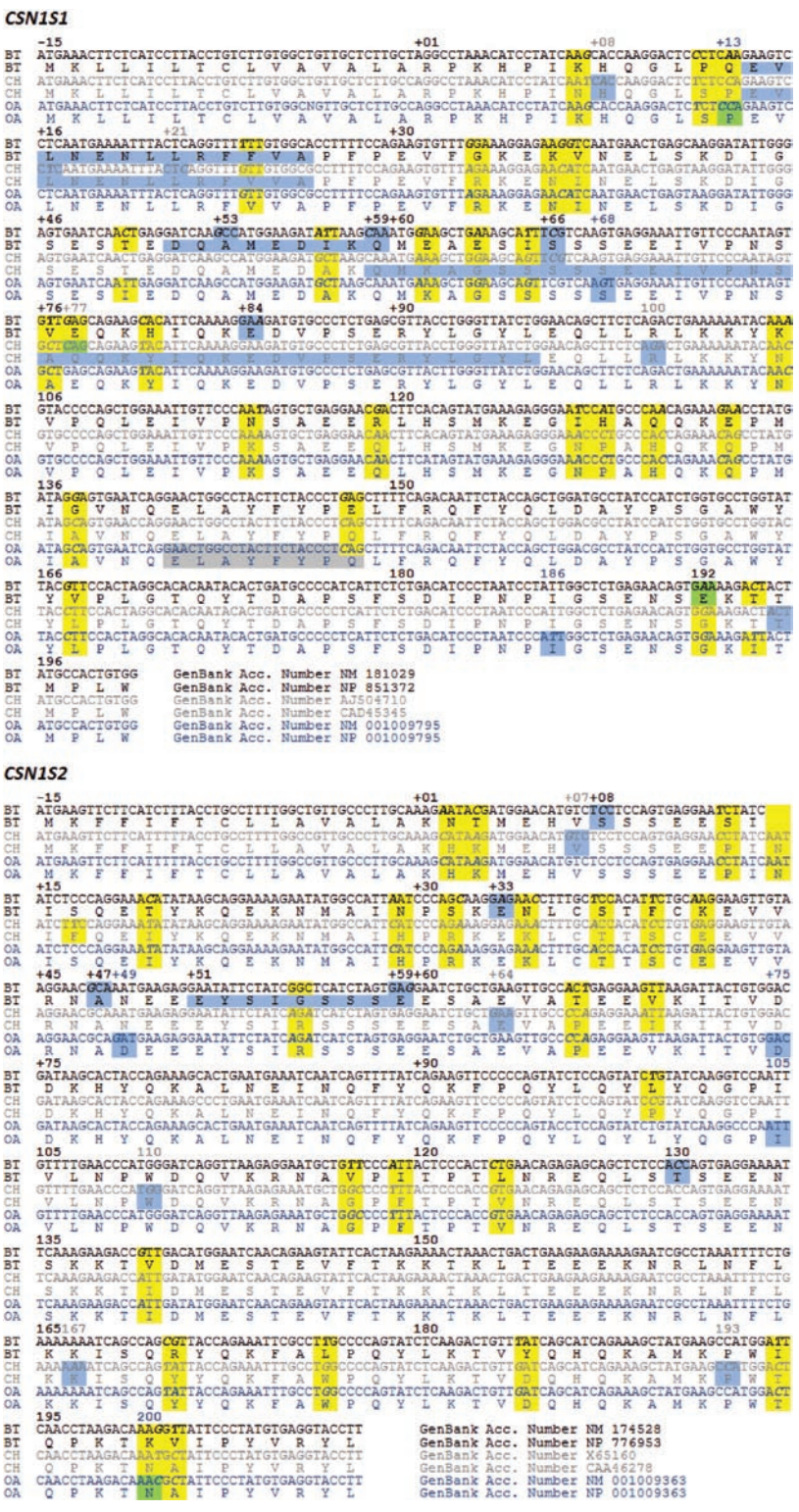

Figure 2a. Alignment of the sequences of *Bos taurus* (BT), *Capra hircus* (CH), and *Ovis aries* (OA) casein genes (lines 1, 3, and 5) and pre-protein (lines 2, 4, and 6). The positions above the first line refer to the protein, starting from the signal peptide (indicated as negative values) to the first AA of the mature protein (+01). Highlighted in yellow: nucleotides and AA differing in *Capra hircus* and/or *Ovis aries* with respect to *Bos taurus*; in blue: nucleotide and/or AA in which intraspecies genetic variations occur; in green: both interspecific and intraspecific variation occur; in gray: missing or conflicting data (AA 141–148 are not available in the ovine reference sequence; AA −9 is not clearly defined from the ovine nucleotide sequence; AA 137 is a controversial inversion with AA 138 in the bovine *CSN2* sequence, see Table 2). Nucleotides affected by interspecific or intraspecific variation are bolded and in italics. *CSN1S1* = α_{s1}-CN gene; *CSN1S2* = α_{s2}-CN gene.

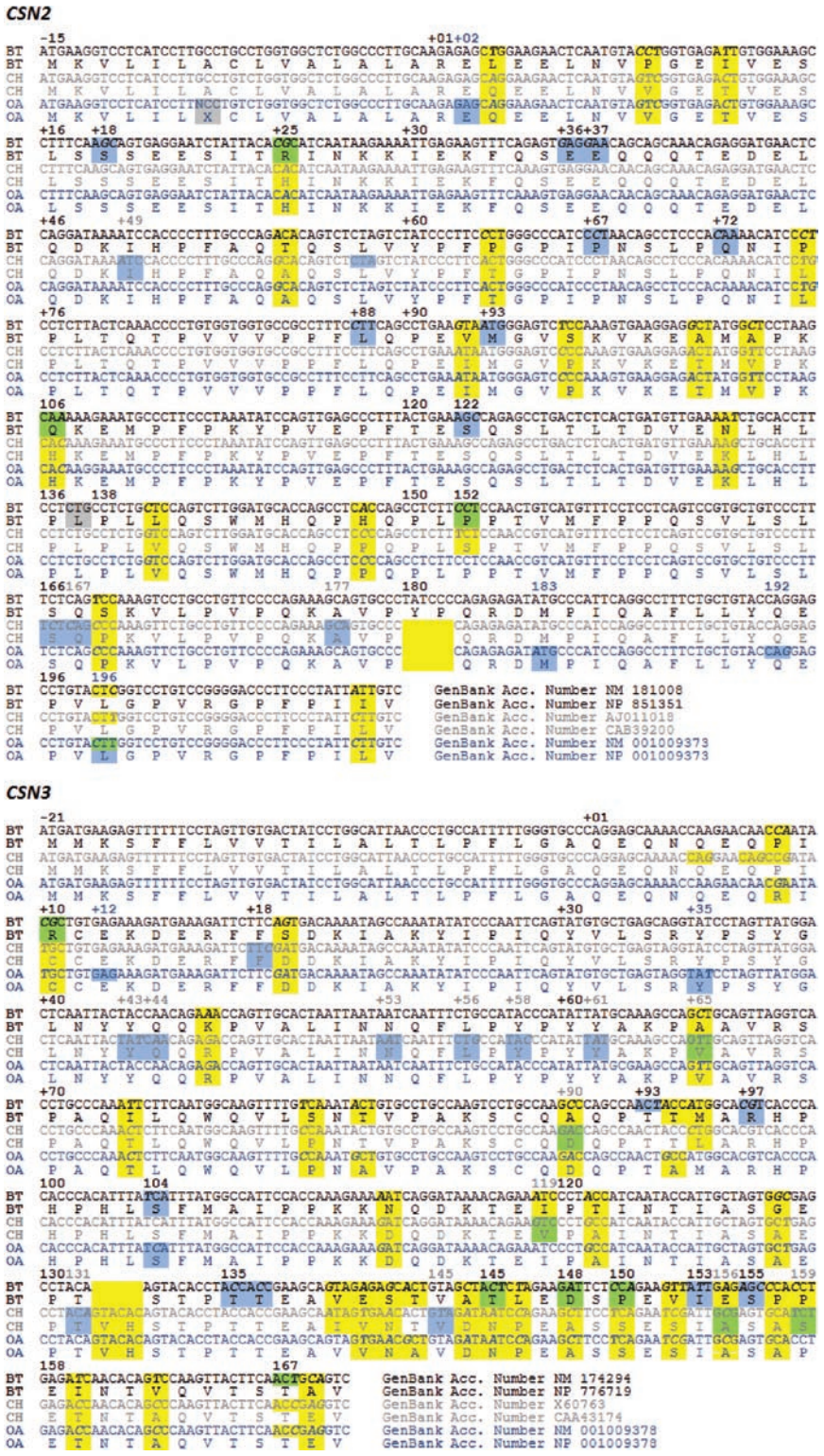

Figure 2b. Alignment of the sequences of *Bos taurus* (BT), *Capra hircus* (CH), and *Ovis aries* (OA) casein genes (lines 1, 3, and 5) and pre-protein (lines 2, 4, and 6). The positions above the first line refer to the protein, starting from the signal peptide (indicated as negative values) to the first AA of the mature protein (+01). Highlighted in yellow: nucleotides and AA differing in *Capra hircus* and/or *Ovis aries* with respect to *Bos taurus*; in blue: nucleotide and/or AA in which intraspecies genetic variations occur; in green: both interspecific and intraspecific variation occur; in gray: missing or conflicting data (AA 141–148 are not available in the ovine reference sequence; AA −9 is not clearly defined from the ovine nucleotide sequence; AA 137 is a controversial inversion with AA 138 in the bovine *CSN2* sequence, see Table 2). Nucleotides affected by interspecific or intraspecific variation are bolded and in italics. *CSN2* = β-CN gene; *CSN3* = κ-CN gene.

differential transcription activity controlled by β-LG A and B–specific promoters, with a 57 and 43% level of expression for *LGB*A* and *LGB*B* promoters, respectively.

More recently, Ganai et al. (2008) detected 50 polymorphisms in the coding, intron, and promoter regions of bovine *LGB* gene, of which 33 had not been described before. The authors concentrated their attention on 8 polymorphisms not in complete linkage disequilibrium with β-LG A and B. One of them (g.-731G > A) had a significant effect on β-LG, the A nt reducing the relative β-LG concentration in animals homozygous for the *LGB*A* variant.

In addition, an extremely weak β-LG B variant band has been observed in Brown Swiss cattle, and the putative responsible allele was named β-LG B* (Kim et al., 1996). The C > A SNP at position 215 bp upstream of the translation initiation site (g.-215C > A) was found to be associated with an abnormally reduced expression of β-LG characterizing this variant (Braunschweig and Leeb, 2006).

As far as *LAA* is concerned, Bleck and Bremel (1993a) identified 3 SNP in Holstein-Friesian within the 5′-flanking region at positions +15, +21, and +54 from the mRNA transcription starting point. The +15 and +21 SNP were in the 5′-untranslated region, whereas the +54 SNP was a silent mutation in the coding region. An A > G SNP differentiated *LAA*(+15)**A* from *LAA*(+15)**B.* The former allele was associated with greater milk, protein, and fat yields, and the latter was associated with greater protein and fat percentage (Bleck and Bremel, 1993b). This SNP was also detected in Taiwan Holstein, Italian Friesian, Italian Red Pied, Swedish Red and White, and Italian Brown (Formaggioni et al., 1999). Voelker et al. (1997) found another SNP located in position −1689 from the transcription start point of *LAA* in complete linkage disequilibrium with *LAA*(+15).

MILK PROTEIN VARIANTS AND HUMAN NUTRITION

It is noteworthy that milk protein polymorphisms are involved in human nutrition in various ways. Three crucial aspects include i) the hypoallergenic properties of particular types of milk, ii) the release of peptides with biological functions from milk proteins, and iii) the coevolution of bovine milk protein variants and human lactose tolerance.

Hypoallergenic Milk

Most milk proteins are potential allergens, mainly α_{s1}-CN, α_{s2}-CN, and β-LG, which are missing in human milk (EFSA, 2004; Crittenden and Bennett, 2005). The occurrence of alleles associated with null or faint content of those proteins might be exploited for the production of milk with particular nutritional qualities; that is, hypoallergenic properties. In this regard, the bovine *CSN1S1*G* (Rando et al., 1998) might be exploited for the production of milk with reduced expression of the specific protein. This is also true for the numerous goat CN alleles associated with null or reduced CN expression (Rando et al., 2000), which deserve a brief description. Of the 45 goat CN alleles that have been identified (summarized in Caroli et al., 2006; Chessa et al., 2008b), 8 and 4 alleles have been associated with null and reduced content, respectively, of the specific calcium-sensitive α_{s1}-CN, α_{s2}-CN, and β-CN. Moreover, a significant variation has been found in milk CN content between the 2 groups of goat *CSN3* variants characterized by different isoelectric points (Chiatti et al., 2007).

Comparison among cattle, goat, and sheep CN coding sequences (Figures 2a and 2b) highlights the lower within-species polymorphism characterized in the ovine species (Amigo et al., 2000; Chessa et al., 2003), with a total of only 14 mutations identified in *Ovis aries* versus 31 (plus 3 deletions) in *Bos taurus* and 31 (plus 2 deletions) in *Capra hircus*. Two deletions affect the same AA in *Bos taurus* and *Capra hircus*, involving AA 14–26 of α_{s1}-CN in bovine *CSN1S1*A* and goat *CSN1S1*G* variant, respectively (Rando et al., 2000). The associated interspecific and intraspecific variations are highlighted in green in Figures 2a and 2b. They occur only at the calcium-sensitive CN in *Ovis aries*, whereas they also affect *CSN3* in *Bos taurus* and *Capra hircus*. Most variations affect the first half of the mature protein in *CSN1S1* and *CSN1S2*, are more uniformly distributed in *CSN2*, and are clearly located in the second half of the *CSN3* gene, coding for CMP with an important possible influence on the biological actions of this peptide.

Besides selecting for milk with null or reduced content of a specific protein, another possibility for producing hypoallergenic milk could involve genetic differences among epitopes, which are short fragments widely spread throughout hydrophobic parts of the protein molecules. Epitopes on milk proteins comprise highly conserved sequences responsible for IgE cross-reactivity with corresponding milk proteins of other mammals, including humans (Wal, 2004). The genetic variation might affect the IgE-binding epitope structure of milk proteins, and as a consequence, milk produced by different genotypes could give different allergenic reactions. Lisson and Erhardt (2008) demonstrated with an in silico study that different epitopes occurring at bovine milk proteins are modified within the genetic variants, as has also been found in the caprine species (Chessa et

al., 2008a). Focusing on the most allergenic fractions, α_{s1}-CN and β-LG, interesting differences resulting from genetic variations were observed for *CSN1S1*A* and *CSN1S1*C* compared with *CSN1S1*B*, whereas 7 AA exchanges were found within 5 epitopes of β-LG in 6 rare *LGB* variants, compared with *LGB*B* (Lisson and Erhardt, 2008).

Biopeptides

In recent years it has been recognized that dietary proteins provide a rich source of biologically active peptides. Biopeptides have been defined as specific protein fragments that have a positive effect on body functions or conditions and might ultimately influence health (Kitts and Weiler, 2003). Such peptides are inactive within the sequence of the parent protein and can be released by enzymatic proteolysis during gastrointestinal digestion or food processing (Fitzgerald and Murray, 2006; Korhonen and Pihlanto, 2006). Caseins represent a reservoir for a wide variety of bioactive peptides, minor regulatory compounds with hormone-like activity, which could affect milk nutritional value (Meisel, 1998; Lorenzini et al., 2007).

The biological activity of peptides released from milk protein digestion might be affected by AA exchanges or deletions resulting from gene mutations. As an example, variant *CSN2*A*^{2} and *CSN2*A*^{3} differ from *CSN2*A*^{1}, *CSN2*B*, and *CSN2*C* for the presence of a proline instead of a histidine at position 67 of the mature protein. Histidine$_{67}$ determines the enzymatic cleavage of the peptide bond releasing β-casomorphin-7, which has opioid properties resulting in an immune suppressant influence most probably implicated in the etiology of type 1 diabetes. It has been suggested that consumption of milk from His$_{67}$-carrying cows is positively and significantly correlated with diabetes incidence because of the release of β-casomorphin-7 (Elliott et al., 1999). Another trial on β-casomorphin-8 on the inhibition of muscle contraction in guinea pigs suggested a difference, even if not statistically significant, between β-casomorphin-8-His and β-casomorphin-8-Pro, the first peptide showing a greater inhibition on muscle contraction (Hartwig et al., 1997). It has also been suggested that a high consumption of His$_{67}$ milk increases the risk of ischemic heart disease (McLachlan, 2001), sudden infant death syndrome (Sun et al., 2003), and the aggravation of symptoms associated with schizophrenia and autism (reviewed in Knivsber et al., 2001) and might correlate with milk allergy (Chatchatee et al., 2001a, b) in humans.

Because high consumption of milk carrying His$_{67}$ (namely *CSN2*A*^{1}, *CSN2*B*, and *CSN2*C*) has been alleged to affect human health by increasing the risk of diabetes and heart disease, an increase in the frequency of alleles or haplotypes coding for Pro$_{67}$ at β-CN by selective breeding has been suggested by Nilsen et al. (2009). However, Hernández-Ledesma et al. (2004) demonstrated the formation of an angiotensin-I-converting enzyme–inhibitory peptide specific to the bovine *CSN2*A*^{1} variant that is not present in other β-CN alleles. Finally, caution is needed because of conflicting results on the potential health effect of β-casomorphins and related peptides recently reviewed in an European Food Safety Authority report (EFSA, 2009). In particular, a cause–effect relationship between the oral intake of β-casomorphin-7 or related peptides and etiology of any suggested diseases cannot be established, and consequently, a formal European Food Safety Authority risk assessment of food-derived peptides is not recommended. This does not exclude, in our opinion, that milk produced from cows carrying particular genotypes might be suggested to be more suitable for human nutrition in specific pathology situations.

Despite the many studies and intensive debate on the so-called *CSN2 A*^{2} milk (with Pro$_{67}$ instead of His$_{67}$, including *CSN2 A*^{3}), investigations on other genetic polymorphisms potentially affecting milk protein peptides are scarce. Two recent studies on biopeptides that took into account the effect of genetic polymorphisms are described in the following.

Weimann et al. (2009) investigated peptides derived from the genetic variants *A*, *B*, *C*, *E*, *F*^{1}, *F*^{2}, *G*^{1}, *G*^{2}, *H*, *I*, and *J* of bovine *CSN3* for their antihypertensive activities. Amino acid sequences of the *CSN3* variants were analyzed in silico to detect potential inhibitory peptides against angiotensin-I-converting enzyme. Some *CSN3* variants carried the following exclusive peptides whose angiotensin-I-converting enzyme–inhibitory activity was determined: ASP (within *CSN3*B*), AHHP (*CSN3*C*), VSP (*CSN3*F*^{1}), and ACHP (*CSN3*G*^{2}).

Among biopeptides, phosphopeptides are strongly phosphorylated peptides known to exert an effect on calcium metabolism but also on other minerals (Bouhallab and Bouglé, 2004). Consumption of high concentrations of calcium in early life contributes to the development of maximal bone density, which, in turn, can prevent osteoporosis in later life. The high bioavailability of calcium from milk and dairy products has, in part, been attributed to the production of caseinophosphopeptides with different levels of phosphorylation (Fitzgerald, 1998). Caroli et al. (2009) investigated the effects of 4 selected casein peptides on osteoblast mineralization in vitro. The chosen peptides were related to different casein genetic variants, in particular *CSN2*C* and *CSN1S2*C* versus the other *CSN2* and *CSN1S2* variants, respectively. The authors suggested that the distinct peptides might differentially affect calcium de-

position in the extracellular matrix and that the genetic variation is involved in their differential effect.

Coevolution of Bovine Milk Genetic Variants and Human Lactose Tolerance

A gene-culture coevolution between cattle milk protein genes and human lactase genes has been recently highlighted (Beja-Pereira et al., 2003). Milk from domestic cows has been a valuable food source for over 8,000 yr, especially in lactose-tolerant human societies that have exploited dairy breeds. Some human populations (e.g., northern Europe) have the genetically determined ability to digest lactose by the action of persistent lactase enzyme in adulthood, thereby benefiting from the rich food resources in cows' milk. The geographic patterns of the variation in genes encoding the 6 most important milk proteins were studied in 70 native European cattle breeds (Beja-Pereira et al., 2003). The authors found a substantial geographic correspondence between high diversity in cattle milk genes, location of the European Neolithic cattle farming sites (>5,000 yr ago), and present-day lactose tolerance in Europeans. The authors proposed that since the Neolithic times, there has been a gene-culture coevolution between the domestic cattle and human culture driven by the advantage conferred by milk consumption. This led to the maintenance of larger herds and selection for increased milk yield and altered milk protein composition. The gene-culture coevolution between cattle milk protein genes and human lactase genes described is an impressive proof of the nonrandom occurrence of milk protein genetic variation over the ages.

CONCLUSIONS

The genomic and proteomic approach is a useful tool for gaining a better understanding of both how selection has modified the ruminants' germplasm at the level of milk protein structural genes and how animal breeding could better exploit the genetic reservoirs of the different genes and breeds. The great variation highlighted in the genes, proteins, and peptides that are so important for dairy production is a crucial element in providing milk with different properties at the level of the protein system. This is important for possible technological use and for the nutraceutical value of dairy products. Designing milk with different protein structures appropriate for its specific use is becoming more and more feasible for breeders and is an important task for animal geneticists.

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REFERENCES

Aleandri, R., L. G. Buttazzoni, J. C. Schneider, A. Caroli, and R. Davoli. 1990. The effects of milk protein polymorphisms on milk components and cheese-producing ability. J. Dairy Sci. 73:241–255.

Alexander, L. J., A. F. Stewart, A. G. Mackinlay, T. V. Kapelinskaya, T. M. Tkach, and S. I. Gorodetsky. 1988. Isolation and characterization of the bovine kappa-casein gene. Eur. J. Biochem. 178:395–401.

Amigo, L., I. Recio, and M. Ramos. 2000. Genetic polymorphism of ovine milk proteins: Its influence on technological properties of milk—A review. Int. Dairy J. 10:135–149.

Aschaffenburg, R., and J. Drewry. 1957. Genetics of the β-lactoglobulins of cows' milk. Nature 180:376–378.

Bai, W. L., R. H. Yin, S. J. Zhao, Y. C. Zheng, J. C. Zhong, and Z. H. Zhao. 2008. Short communication: Characterization of a κ-casein genetic variant in the Chinese yak, *Bos grunniens*. J. Dairy Sci. 91:1204–1208.

Barroso, A., S. Dunner, and J. Canon. 1999. A multiplex PCR-SSCP test to genotype bovine beta-casein alleles A^1, A^2, A^3, B, and C. Anim. Genet. 30:322–323.

Bawden, W. S., and K. R. Nicholas. 1999. Molecular genetics of milk production. Pages 539–576 in The Genetics of Cattle. R. Fries and A. Ruvinsky, ed. CABI Publishing, New York, NY.

Beja-Pereira, A., G. Erhardt, C. Matos, L. Gama, and N. Ferrand. 2002. Evidence of a geographical cline of casein haplotypes in Portuguese cattle breeds. Anim. Genet. 33:295–300.

Beja-Pereira, A., G. Luikart, P. R. England, D. G. Bradley, O. C. Jann, G. Bertorelle, A. T. Chamberlain, T. P. Nunes, S. Metodiev, N. Ferrand, and G. Erhardt. 2003. Gene-culture coevolution between cattle milk protein genes and human lactase genes. Nat. Genet. 35:311–313.

Bell, K., K. E. Hopper, and H. A. McKenzie. 1981. Bovine α-lactalbumin C and αs1-, β-, and κ-caseins of Bali (Banteng) cattle, *Bos* (Bibos) *javanicus*. Aust. J. Biol. Sci. 34:149–159.

Bleck, G. T., and R. D. Bremel. 1993a. Sequence and single-base polymorphisms of the bovine α-lactalbumin 5′-flanking region. Gene 126:213–218.

Bleck, G. T., and R. D. Bremel. 1993b. Correlation of the α-lactalbumin (+15) polymorphism to milk production and milk composition of Holsteins. J. Dairy Sci. 76:2292–2298.

Boettcher, P. J., A. Caroli, A. Stella, S. Chessa, E. Budelli, F. Canavesi, S. Ghiroldi, and G. Pagnacco. 2004. Effects of casein haplotypes on production traits in Italian Holstein and Brown cattle. J. Dairy Sci. 87:4311–4317.

Bonsing, J., and A. G. Mackinlay. 1987. Recent studies on nucleotide sequences encoding the caseins. J. Dairy Res. 54:447–461.

Bouhallab, S., and D. Bouglé. 2004. Biopeptides of milk: Caseinophosphopeptides and mineral bioavailability. Reprod. Nutr. Dev. 44:493–498.

Braunschweig, M., C. Hagger, G. Stranzinger, and Z. Puhan. 2000. Associations between casein haplotypes and milk production traits of Swiss Brown cattle. J. Dairy Sci. 83:1387–1395.

Braunschweig, M. H., and T. Leeb. 2006. Aberrant low expression level of bovine *β-lactoglobulin* is associated with a C to A transversion in the BLG promoter region. J. Dairy Sci. 89:4414–4419.

Caroli, A., P. Bolla, E. Budelli, G. Barbieri, and P. Leone. 2000. Effect of k-casein E allele on clotting aptitude of Italian Friesian milk. Zoot. Nutr. Anim. 3:127–130.

Caroli, A., O. Bulgari, S. Chessa, D. Cocchi, and G. Tulipano. 2009. In vitro evaluation of caseinophosphopeptides from different genetic variants on bone mineralization. Ital. J. Anim. Sci. 8(Suppl. 2): 42–44.

Caroli, A., S. Chessa, F. Chiatti, D. Rignanese, B. Meléndez, R. Rizzi, and G. Ceriotti. 2008. Short communication: Carora cattle show high variability in α_{s1}-casein. J. Dairy Sci. 91:354–359.

Caroli, A., F. Chiatti, S. Chessa, D. Rignanese, P. Bolla, and G. Pagnacco. 2006. Focusing on the goat casein gene complex. J. Dairy Sci. 89:3178–3187.

Cerbulis, J., and H. M. Farrell Jr.. 1975. Composition of milks of dairy cattle. I. Protein, lactose, and fat contents and distribution of protein fraction. J. Dairy Sci. 58:817–827.

Ceriotti, G., S. Chessa, G. Bramante, P. Bolla, E. Pieragostini, and A. Caroli. 2004b. Un approccio molecolare alla tracciabilità dei prodotti lattiero-caseari caprini. Sci. Tecn. Latt. Cas. 55:251–262.

Ceriotti, G., D. Marletta, A. Caroli, and G. Erhardt. 2004a. Milk protein loci in taurine (*Bos taurus*) and zebu (*Bos indicus*) populations bred in hot climate. J. Anim. Breed. Genet. 121:404–415.

Chatchatee, P., K. M. Jarvinen, L. Bardina, K. Beyer, and H. A. Sampson. 2001a. Identification of IgE- and IgG-binding epitopes on alpha(s1)-casein: Differences in patients with persistent and transient cow's milk allergy. J. Allergy Clin. Immunol. 107:379–383.

Chatchatee, P., K. M. Jarvinen, L. Bardina, L. Vila, K. Beyer, and H. A. Sampson. 2001b. Identification of IgE and IgG binding epitopes on beta- and kappa-casein in cow's milk allergic patients. Clin. Exp. Allergy 31:1256–1262.

Chen, S. Y., V. Costa, M. Azevedo, M. Baig, N. Malmakov, G. Luikart, G. Erhardt, and A. Beja-Pereira. 2008. Short communication: New alleles of the bovine kappa-casein gene revealed by resequencing and haplotype inference analysis. J. Dairy Sci. 91:3682–3686.

Chessa, S., E. Budelli, A. Caroli, and E. Pieragostini. 2003. Variabilità delle lattoproteine nella specie ovina: Stato dell'arte e prospettive. Sci. Tecn. Latt. Cas. 54:35–43.

Chessa, S., F. Chiatti, G. Ceriotti, A. Caroli, C. Consolandi, G. Pagnacco, and B. Castiglioni. 2007. Development of a SNP genotyping microarray platform for the identification of bovine milk protein genetic polymorphisms. J. Dairy Sci. 90:451–464.

Chessa, S., F. Chiatti, D. Rignanese, G. Ceriotti, A. M. Caroli, and G. Pagnacco. 2008a. Analisi *in silico* delle sequenze caseiniche caprine. Sci. Tecn. Latt. Cas. 59:71–79.

Chessa, S., D. Rignanese, J. Küpper, G. Pagnacco, G. Erhardt, and A. Caroli. 2008b. Short communication*:* The β-casein (*CSN2*) silent allele *C1* is highly spread in goat breeds. J. Dairy Sci. 91:4433–4436.

Chiatti, F., S. Chessa, P. Bolla, G. Cigalino, A. Caroli, and G. Pagnacco. 2007. Effect of κ-casein polymorphism on milk composition in Orobica goat. J. Dairy Sci. 90:1962–1966.

Comin, A., M. Cassandro, S. Chessa, M. Ojala, R. Dal Zotto, M. De Marchi, P. Carnier, L. Gallo, G. Pagnacco, and G. Bittante. 2008. Effects of composite β- and κ-casein genotypes on milk coagulation, quality, and yield traits in Italian Holstein cows. J. Dairy Sci. 91:4022–4027.

Crittenden, R. G., and L. E. Bennett. 2005. Cow's milk allergy: A complex disorder. J. Am. Coll. Nutr. 24:582S–591S.

Damiani, G., E. Budelli, S. Florio, A. Caroli, and G. Pagnacco. 2000a. Polymorphism of κ-casein SINE Bov-A2 and CYP21-hydroxylase in some bovine breeds. Zoot. Nutr. Anim. 3:145–148.

Damiani, G., A. Caroli, P. Leone, E. Budelli, and S. Florio. 2001. Effect of Bov-A2 Sine elements on quantitative traits. Pages 49–51 in Proc. XIV Natl. Congr. ASPA, Florence, Italy. University of Florence Press, Florence, Italy.

Damiani, G., L. Ferretti, G. Rognoni, and V. Sgaramella. 1990. Restriction fragment length polymorphism analysis of the kappa-casein locus in cattle. Anim. Genet. 21:107–114.

Damiani, G., S. Florio, E. Budelli, P. Bolla, and A. Caroli. 2000b. Single nucleotide polymorphisms (SNPs) within Bov-A2 SINE in the second intron of bovine and buffalo κ-casein (CSN3) gene. Anim. Genet. 31:277–279.

Damiani, G., F. Pilla, P. Leone, and S. Cacciò. 1992. Direct sequencing and bidirectional allele specific polymerase chain reaction of the bovine beta-casein B variant. Anim. Genet. 23:561–565.

David, V. A., and A. H. Deutch. 1992. Detection of bovine α_{s1}-casein genomic variants using the allele-specific polymerase chain reaction. Anim. Genet. 23:425–429.

Di Stasio, L., and P. Mariani. 2000. The role of protein polymorphism in the genetic improvement of milk production. Zoot. Nutr. Anim. 26:69–90.

Di Stasio, L., and P. Merlin. 1979. Polimorfismi biochimici del latte nella razza bovina Grigio Alpina. Riv. Zoot. Vet. 2:64–67.

EFSA (European Food Safety Authority). 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of goats' milk protein as a protein source for infant formulae and follow-on formulae. EFSA J. 30:1–15.

EFSA (European Food Safety Authority). 2009. Review of the potential health impact of β-casomorphins and related peptides. EFSA Sci. Rep. 231:1–107.

Elliott, R. B., D. P. Harris, J. P. Hill, N. J. Bibby, and H. E. Wasmuth. 1999. Type I (insulin-dependent) diabetes mellitus and cow milk: Casein variant consumption. Diabetologia 42:292–296.

Erhardt, G. 1996. Detection of a new κ-casein variant in the milk of Pinzgauer cattle. Anim. Genet. 27:105–107.

Erhardt, G., and A. Eggen. 1990. Untersuchungsmöglichkeiten der Milchproteine mittels Protein- und DNS-Analyse und deren Bedeutung bei züchterischen Fragestellungen. Landwirtschaft Schweiz 3:181–186.

Erhardt, G., E. M. Prinzenberg, J. Buchberger, H. Krick-Saleck, I. Krause, and M. Miller. 1997. Bovine κ-casein G detection, occurrence, molecular genetic characterization, genotyping and coagulation properties. Pages 328–329 in Proc. IDF "Milk Protein Polymorphism Seminar II." International Dairy Federation, Brussels, Belgium.

Erhardt, G., and B. Senft. 1991. Nutzungsmöglichkeiten der genetisch bedingten Varianten der Rindermilch im Rahmen der Milchleistungsprüfung. Pages 20–22 in Performance Recording of Animals: State of the Art, 1990. P. Gaillon and Y. Chabert, ed. Springer Netherlands, Pudoc, Wageningen, the Netherlands.

Farrell, H. M. Jr., R. Jimenez-Flores, G. T. Bleck, E. M. Brown, J. E. Butler, L. K. Creamer, C. L. Hicks, C. M. Hollar, K. F. Ng-Kwai-Hang, and H. E. Swaisgood. 2004. Nomenclature of the proteins of cows' milk—Sixth revision. J. Dairy Sci. 87:1641–1674.

Ferretti, L., P. Leone, and V. Sgaramella. 1990. Long range restriction analysis of the bovine casein genes. Nucleic Acids Res. 18:6829–6833.

Fitzgerald, R. J. 1998. Potential uses of caseinophosphopeptides. Int. Dairy J. 8:451–457.

Fitzgerald, R. J., and B. A. Murray. 2006. Bioactive peptides and lactic fermentations . Int. J. Dairy Technol. 59:118–125.

Folch, J. M., P. Dovč, and J. F. Medrano. 1999. Differential expression of bovine β-lactoglobulin A and B promoter variants in transiently transfected HC11 cells. J. Dairy Res. 66:537–544.

Formaggioni, P., A. Summer, M. Malacarne, and P. Mariani. 1999. Milk protein polymorphism: Detection and diffusion of the genetic variants in *Bos* genus. Ann. Fac. Med. Vet. Un. Parma. XIX:127–165.

Freyer, G., Z. Liu, G. Erhardt, and L. Panicke. 1999. Casein polymorphism and relation between milk production traits. J. Anim. Breed. Genet. 116:87–97.

Ganai, N. A., H. Bovenhuis, J. A. M. van Arendonk, and M. H. P. W. Visker. 2008. Novel polymorphisms in the bovine β-lactoglobulin gene and their effects on β-lactoglobulin protein concentration in milk. Anim. Genet. 40:127–133.

Gatesy, J., C. Hayashi, M. A. Cronin, and P. Arctander. 1996. Evidence from milk casein genes that cetaceans are close relatives of hippopotamid artiodactyls. Mol. Biol. Evol. 13:954–963.

Gorodetskiĭ, S. I., and A. S. Kaledin. 1987. Nucleotide sequence of the cDNA of kappa casein in cows. Genetika 23:596–604.

Grosclaude, F. 1988. Le polymorphisme génétique des principales lactoprotéines bovines. Relations avec la quantité, la composition et les aptitudes fromagères du lait. INRA Prod. Anim. 1:5–17.

Han, S. K., Y. C. Shin, and H. D. Byun. 2000. Biochemical, molecular and physiological characterization of a new β-casein variant detected in Korean cattle. Anim. Genet. 31:49–51.

Hartwig, A., H. Teschemacher, W. Lehmann, M. Gauly, and G. Erhardt. 1997. Influence of genetic polymorphism in bovine milk on the occurrence of bioactive peptides. Pages 459–460 in Proc. IDF "Milk Protein Polymorphism Seminar II." International Dairy Federation, Brussels, Belgium..

Hayes, H., and E. Petit. 1993. Mapping of the β-lactoglobulin gene and of an immunoglobulin M heavy chain-like sequence in homologous cattle, sheep, and goat chromosomes. Mamm. Genome 4:207–210.

Hayes, H., E. Petit, C. Bouniol, and P. Popescu. 1993. Localisation of the alpha-S2-casein gene (CASAS2) to the homologous cattle, sheep and goat chromosome 4 by in situ hybridisation. Cytogenet. Cell Genet. 64:282–285.

Heck, J. M. L., A. Schennink, H. J. F. van Valenberg, H. Bovenhuis, M. H. P. W. Visker, J. A. M. van Arendonk, and A. C. M. van Hooijdonk. 2009. Effects of milk protein variants on the protein composition of bovine milk. J. Dairy Sci. 92:1192–1202.

Hernández-Ledesma, B., L. Amigo, M. Ramos, and I. Recio. 2004. Angiotensin converting enzyme inhibitory activity in commercial fermented products: Formation of peptides under simulated gastrointestinal digestion. J. Agric. Food Chem. 52:1504–1510.

Ibeagha-Awemu, E. M., P. Kgwatalala, and X. Zhao. 2008. A critical analysis of production-associated DNA polymorphisms in the genes of cattle, goat, sheep, and pig. Mamm. Genome 19:591–617.

Ibeagha-Awemu, E. M., E.-M. Prinzenberg, O. C. Jann, G. Lühken, A. E. Ibeagha, X. Zhao, and G. Erhardt. 2007. Molecular characterization of bovine *CSN1S2B* and extensive distribution of zebu specific milk protein alleles in European cattle. J. Dairy Sci. 90:3522–3529.

Ikonen, T., H. Bovenhuis, M. Ojala, O. Ruottinen, and M. Georges. 2001. Associations between casein haplotypes and first lactation milk production traits in Finnish Ayrshire cows. J. Dairy Sci. 84:507–514.

Jann, O., G. Ceriotti, A. Caroli, and G. Erhardt. 2002b. A new variant in exon VII of bovine β-casein gene (*CSN2*) and its distribution among European cattle breeds. J. Anim. Breed. Genet. 119:65–68.

Jann, O., E. Ibeagha-Awemu, C. Özbeyaz, P. Zaragoza, J. L. Williams, P. Ajmone-Marsan, J. A. Lenstra, K. Moazami-Goudarzi, and G. Erhardt. 2004. Geographic distribution of haplotype diversity at the bovine casein locus. Genet. Sel. Evol. 36:243–257.

Jann, O., E.-M. Prinzenberg, H. Brandt, J. L. Williams, P. Ajmone-Marsan, P. Zaragoza, C. Ozbeyaz, and G. Erhardt. 2002a. Intragenic haplotypes at the bovine *CSN1S1* locus. Arch. Tierz. 45:13–21.

Kim, J. S., M. Braunschweig, and Z. Puhan. 1996. Occurrence of extreme ratio of *β*-lactoglobulin variants A and B in Swiss Brown cattle quantified by capillary electrophoresis. Milchwissenschaft 51:435–438.

Kitts, D. D., and K. Weiler. 2003. Bioactive proteins and peptides from food sources: Applications of bioprocesses used in isolation and recovery. Curr. Pharm. Des. 9:1309–1323.

Knivsber, A. M., K. L. Reichelt, and M. Nodland. 2001. Reports on dietary intervention in autistic disorders. Nutr. Neurosci. 4:25–37.

Korhonen, H., and A. Pihlanto. 2006. Bioactive peptides: Production and functionality. Int. Dairy J. 16:945–960.

Krause, I., J. Buchberger, G. Weiß, and H. Klostermeyer. 1988. Screening methods for genetic variants of milk proteins. Pages 171–173 in Milk Proteins: Nutritional, Clinical, Functional and Technological Aspects. C. A. Barth and E. Schlimme, ed. Steinkopff Verlag, Darmstadt, Germany.

Kühn, C., G. Freyer, R. Weikard, T. Goldammer, and M. Schwerin. 1999. Detection of QTL for milk production traits in cattle by application of a specifically developed markermap of BTA6. Anim. Genet. 30:333–340.

Lemay, D. G., D. J. Lynn, W. F. Martin, M. C. Neville, T. M. Casey, G. Rincon, E. V. Kriventseva, W. C. Barris, A. S. Hinrichs, A. J. Molenaar, K. S. Pollard, N. J. Maqbool, K. Singh, R. Murney, E. M. Zdobnov, R. L. Tellam, J. F. Medrano, J. B. German, and M. Rijnkels. 2009. The bovine lactation genome: Insights into the evolution of mammalian milk. Genome Biol. 10:R43.

Lien, S., L. Gomez-Raya, T. Steine, E. Fimland, and S. Rogne. 1995. Associations between casein haplotypes and milk yield traits. J. Dairy Sci. 78:2047–2056.

Lien, S., J. Kantanen, I. Olsaker, L. E. Holm, E. Eythorsdottir, K. Sandberg, B. Dalsgaid, and S. Adalsteinsson. 1999. Comparison of milk protein allele frequencies in Nordic cattle breeds. Anim. Genet. 30:85–91.

Lien, S., and S. Rogne. 1993. Bovine casein haplotypes: Number, frequencies and applicability as genetic markers. Anim. Genet. 24:373–376.

Lisson, M., and G. Erhardt. 2008. Allergenic potential of bovine milk proteins with regards to genetic variants. Invited seminar. University of Brescia, Brescia, Italy.

Lorenzini, E., S. Chessa, F. Chiatti, A. Caroli, and G. Pagnacco. 2007. Peptidi bioattivi di latte e derivati. Sci. Tecn. Latt. Cas. 58:113–156.

Losi, G., G. B. Castagnetti, L. Grazia, C. Zambonetti, P. Mariani, and V. Russo. 1973. Influenza delle varianti genetiche della caseina κ sulla formazione e sulle caratteristiche della cagliata. Sci. Tech. Alim. 3:373–374.

Lühken, G., A. Caroli, E. M. Ibeagha-Awemu, and G. Erhardt. 2009. Characterization and genetic analysis of bovine α_{s1}-casein I variant. Anim. Genet. 40:479–485.

Lum, L. S., P. Dovč, and J. F. Medrano. 1997. Polymorphisms of bovine *β*-lactoglobulin promoter and differences in the binding affinity of activator protein-2 transcription factor. J. Dairy Sci. 80:1389–1397.

Mahé, M. F., G. Miranda, R. Queval, A. Bado, P. S. Zafindrajona, and F. Grosclaude. 1999. Genetic polymorphism of milk proteins in African *Bos taurus* and *Bos indicus* populations: Characterization of variants α_{s1}-Cn H and κ-Cn J. Genet. Sel. Evol. 31:239–253.

Malkoski, M., S. G. Dashper, N. M. O'Brien-Simpson, G. H. Talbo, M. Macris, K. J. Cross, and E. C. Reynolds. 2001. Kappacin, a novel antibacterial peptide from bovine milk. Antimicrob. Agents Chemother. 45:2309–2315.

Mariani, P. 1983. Sulla presenza di una terza k-caseina nel latte di vacche di razza Bruna. Sci. Tecn. Latt. Cas. 34:174–181.

Mariani, P., G. Losi, V. Russo, G. B. Castagnetti, L. Grazia, D. Morini, and E. Fossa. 1976. Prove di caseificazione con latte caratterizzato dalle varianti A e B delle κ-caseina nella produzione del formaggio Parmigiano-Reggiano. Sci. Tecn. Latt. Cas. 27:208–227.

Martin, P., M. Szymanowska, L. Zwierzchowski, and C. Leroux. 2002. The impact of genetic polymorphisms on the protein composition of ruminants milks. Reprod. Nutr. Dev. 42:433–459.

McLachlan, C. N. 2001. Beta-casein A1, ischaemic heart disease mortality, and other illnesses. Med. Hypotheses 56:262–272.

Medrano, J. F., and E. Aguilar-Cordova. 1990. Polymerase chain reaction amplification of bovine β-lactoglobulin genomic sequences and identification of genetic variants by RFLP analysis. Anim. Biotechnol. 1:73–77.

Meisel, H. 1998. Overview on milk protein derived peptides. Int. Dairy J. 8:363–373.

Mercier, J.-C., G. Brignon, and B. Ribadeau-Dumas. 1973. Structure primarie de la caseine κ-bovine B. Sequence complete. Eur. J. Biochem. 35:222–235.

Mercier, J.-C., J. M. Chobert, and F. Addeo. 1976. Comparative analysis of the amino acid sequences of caseinomacropeptides from seven species. FEBS Lett. 72:208–214.

Mercier, J. C., and J. L. Vilotte. 1993. Structure and function of milk protein genes. J. Dairy Sci. 76:3079–3098.

Miranda, G., P. Anglade, M. F. Mahé, and G. Erhardt. 1993. Biochemical characterization of the bovine genetic k-casein C and E variants. Anim. Genet. 24:27–31.

Moazami-Goudarzi, K., D. M. A. Belemsaga, G. Ceriotti, D. Laloë, F. Fagbohoun, N'T. Kouagou, I. Sidibè, V. Codjia, C. Crimella, F. Grosclaude, and S. M. Tourè. 2001. Caractérisation de la race

bovine Somba à l'aide de marqueurs molécolaires. Rev. Elev. Med. Vet. Pays Trop. 54:129–138.

Mohr, U., D. Koczan, D. Linder, G. Hobom, and G. Erhardt. 1994. A single point mutation results in A allele-specific exon skipping in the bovine α_{S1}-casein mRNA. Gene 143:187–192.

Nilsen, H., H. G. Olsen, B. Hayes, E. Sehested, M. Svendsen, T. Nome, T. Meuwissen, and S. Lien. 2009. Casein haplotypes and their association with milk production traits in Norwegian Red cattle. Genet. Sel. Evol. 41:24.

Olsen, H. G., S. Lien, M. Gautier, H. Nilsen, A. Roseth, P. R. Berg, K. K. Sundsaasen, M. Svendsen, and T. H. E. Meuwissen. 2005. Mapping of a milk production quantitative trait locus to a 420-kb region on bovine chromosome 6. Genetics 169:275–283.

Popescu, C. P., S. Long, P. Riggs, J. Womack, S. Schmutz, R. Fries, and D. S. Gallagher. 1996. Standardization of cattle karyotype nomenclature: Report of the committee for the standardization of the cattle karyotype. Cytogenet. Cell Genet. 74:259–261.

Prinzenberg, E.-M., S. Hiendleder, T. Ikonen, and G. Erhardt. 1996. Molecular genetic characterization of new bovine κ-casein alleles *CSN3-F* and *CSN3-G* and genotyping by PCR-RFLP. Anim. Genet. 27:347–349.

Prinzenberg, E.-M., H. Jianlin, and G. Erhardt. 2008. Genetic variation in the κ-casein gene (*CSN3*) of chinese yak (*Bos grunniens*) and phylogenetic analysis of *CSN3* sequences in the genus *Bos.* J. Dairy Sci. 91:1198–1203.

Prinzenberg, E. M., I. Krause, and G. Erhardt. 1999. SSCP analysis at the bovine *CSN3* locus discriminates six alleles corresponding to known protein variants (*A, B, C, E, F, G*) and 3 new DNA polymorphisms (*H, I,* A^I). Anim. Biotechnol. 10:49–62.

Prinzenberg, E. M., C. Weimann, H. Brandt, J. Bennewitz, E. Kalm, M. Schwerin, and G. Erhardt. 2003. Polymorphism of the bovine *CSN1S1* promoter: Linkage mapping, intragenic haplotypes, and effect on milk production traits. J. Dairy Sci. 86:2696–2705.

Rando, A., P. Di Gregorio, L. Ramunno, P. Mariani, A. Fiorella, C. Senese, D. Marletta, and P. Masina. 1998. Characterization of the $CSN1A^G$ allele of the bovine α_{s1}-casein locus by the insertion of a relict of a long interspersed element. J. Dairy Sci. 81:1735–1742.

Rando, A., L. Ramunno, and P. Masina. 2000. Mutations in casein genes. Zoot. Nutr. Anim. 26:105–114.

Rijnkels, M. 2002. Multispecies comparison of the casein gene loci and evolution of casein gene family. J. Mammary Gland Biol. Neoplasia 7:327–345.

Schlieben, S., G. Erhardt, and B. Senft. 1991. Genotyping of bovine κ-casein (κ-CN^A, κ-CN^B, κ-CN^C, κ-CN^E) following DNA sequence amplification and direct sequencing of κ-CN^E PCR product. Anim. Genet. 22:333–342.

Schopen, G. C. B., P. D. Koks, J. A. M. van Arendonk, H. Bovenhuis, and M. H. P. W. Visker. 2009. Whole genome scan to detect quantitative trait loci for bovine milk protein composition. Anim. Genet. 40:524–537.

Seibert, B., G. Erhardt, and B. Senft. 1987. Detection of a new κ-casein variant in cow's milk. Anim. Genet. 18:269–272.

Senocq, D., D. Mollé, S. Pochet, J. Léonil, D. Dupont, and D. Levieux. 2002. A new bovine β-casein genetic variant characterized by a Met93 > Leu93 substitution in the sequence A^2. Lait 82:171–180.

Sulimova, G. E., I. N. Badagueva, and I. G. Udina. 1996. Polymorphism of the κ-casein gene in subfamilies of the Bovidae. Genetika (Moskva) 32:1576–1582.

Sulimova, G. E., S. S. Sokolova, O. P. Semikozova, L. M. Nguet, and E. M. Berberov. 1992. Analysis of DNA polymorphisms of clustered genes in cattle: Casein genes and genes of the major histocompatibility complex (BOLA). Tsitol. I Genetika 26:18–26.

Sun, Z., Z. Zhang, X. Wang, R. Cade, Z. Elmir, and M. Fregly. 2003. Relation of beta-casomorphin to apnea in sudden infant death syndrome. Peptides 24:937–943.

Threadgill, D. W., and J. E. Womack. 1990. Genomic analysis of the major bovine milk proteins genes. Nucleic Acids Res. 18:6935–6942.

Velmala, R. J., H. J. Vilkki, K. T. Elo, D. J. de Koning, and A. V. Mäki-Tanila. 1999. A search for quantitative trait loci for milk production traits on chromosome 6 in Finnish Ayrshire cattle. Anim. Genet. 30:136–143.

Voelker, G. R., G. T. Bleck, and M. B. Wheeler. 1997. Single-base polymorphisms within the 5′-flanking region of the bovine α-lactalbumin gene. J. Dairy Sci. 80:194–197.

Wagner, V., T. Schild, and H. Geldermann. 1994. DNA variants within the 5-flanking regions of milk protein genes. II. The β-lactoglobulin-encoding gene. Theor. Appl. Genet. 89:121–126.

Wal, J. M. 2004. Bovine milk allergenicity. Ann. Allergy Asthma Immunol. 93:2–11.

Ward, T. J., R. L. Honeycutt, and J. N. Derr. 1997. Nucleotide sequence evolution at the kappa-casein locus: Evidence for positive selection within the family *Bovidae.* Genetics 147:1863–1872.

Weimann, C., H. Meisel, and G. Erhardt. 2009. Short communication: Bovine κ-casein variants result in different angiotensin I converting enzyme (ACE) inhibitory peptides. J. Dairy Sci. 92:1885–1888.