Short Communication: Characterization of a κ -Casein Genetic Variant in the Chinese Yak, *Bos grunniens*

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ABSTRACT

A PCR-single strand conformation polymorphism protocol has been developed for rapid genotyping of the yak κ -casein gene. A total of 307 yaks from the Tianzhu White, Jiulong, Maiwa, and Datong breeds in China were genotyped at the κ -casein locus using the protocol developed in the present study. A polymorphism of κ -casein gene exon 4 has been identified in Tianzhu White breed by evaluating genomic DNA. The polymorphic site consists of a single nucleotide substitution G→C at position 362 of the exon 4, resulting in an AA substitution from Arg to Pro at position 121 of the AA sequence and in 2 alleles named, respectively, G and C based on nucleotide 362. The occurrence of allele C in the Tianzhu White breed was high with an allele frequency of 0.15. However, allele C appears to be absent in the yaks from Jiulong, Maiwa, and Datong breeds.

Key words: genetic polymorphism, yak, κ -casein

Yaks live in the area of central Asia highlands, and the yak has been regarded as one of the world's most remarkable domestic animals, because it thrives in conditions of extreme harshness while providing a livelihood for the local herdsmen (Sasaki, 1994; Wiener et al., 2003). Milk and milk products of yak are important for the herdsmen, and in commercial terms, milk is perhaps the most important of the yak products (Wiener et al., 2003). In general terms, the milk yield of yak is relatively low compared with that of dairy cattle, but yak milk is of good quality and contains a greater percentage of protein compared with milk of dairy cattle (Wang and Zou, 1995). Thus, the production and quality of yak milk attract people's attention (Fan et al., 2000). Nowadays, people are attempting to improve the milk

yield while maintaining its quality through breeding technology.

Casein genes are organized into a tightly linked cluster including, in order, α_{s1} -casein, β -casein, α_{s2} -casein, and κ -casein loci (Ferretti et al., 1990; Threadgill and Womack, 1990; Rijnkels et al., 1997). The genetic polymorphisms of casein are of importance, since some variants could be associated with composition and technological properties of milk (Boland et al., 2001; Martin et al., 2002). Among the casein fractions, κ -casein differs from other caseins in its solubility over a broad range of calcium ion concentrations and contains a hydrophilic C-terminal region (Yahyaoui et al., 2003). On the other hand, κ -casein allows the formation and stabilization of milk micelles and determines their size and function (Gutiérrez-Adan et al., 1996).

Several studies have been carried out on genetic variations in the κ -casein gene of cattle, goats, and sheep (Prinzenberg et al., 1999, 2005; Ceriotti et al., 2004; Jann et al., 2004), whereas the yak κ -casein gene polymorphism has been less extensively investigated. Little information was available about genetic variation of yak κ -casein gene until recently, when 2 polymorphic sites were detected by PCR-RFLP analysis in exon 4 by *HindIII*, and intron 4 by *PstI* (Fan et al., 2000). No further information was provided regarding the biochemical characterization of the patterns observed.

The aims of the present work were to 1) describe a method for rapid genotyping of the yak κ -casein and 2) investigate the genetic variation in exon 4.

Blood samples were randomly collected from the following yak breeds: Tianzhuan White ($n = 75$), Jiulong ($n = 71$), Maiwa ($n = 84$), and Datong ($n = 77$). We tried to avoid sampling multiple individuals with common traceable genetic relationships. Genomic DNA was extracted from blood samples by using the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences, Uppsala, Sweden).

Tianzhu White, which is raised in Tianzhu county of Gansu province, has an estimated population of 60,000 individuals (<http://www.fao.org>; accessed July 5, 2007).

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Figure 1. The PCR-single-strand conformation polymorphism-based DNA test for κ -casein gene exon 4 polymorphism. The black and the white dots indicate G and C occurrence, respectively, in the nucleotide sequence. The second band of each single nucleotide polymorphism migrates at the same level of the first one and therefore is not visible in this figure.

Average lactation milk production is about 304 kg in 135 d, and the contents of milk protein and milk fat are approximately 5.2 and 5.5%, respectively.

The Jiulong breed, numbering around 50,000 animals (<http://www.fao.org>; accessed July 5, 2007) belongs to Jiulong county of Sichuan province. Average lactation milk production is about 414 kg in 150 d, and the contents of milk protein and milk fat are approximately 4.9 and 6.9%, respectively.

The Maiwa breed, belonging to Hongyuan county of Sichuan province, numbers around 600,000 animals (<http://www.fao.org>; accessed July 5, 2007). Average lactation milk production is about 365 kg in 150 d, and the contents of milk protein and milk fat are approximately 4.9 and 6.3%, respectively.

The Datong breed was developed by crossbreeding between local populations and wild yak, and there are about 22,000 animals of this breed (<http://www.fao.org>;

Figure 2. Sequence comparison of positions 1 to 483 of the κ -casein sequence from domestic yak (GenBank accession number AF194989) and the codified protein sequence (first and second sequence, respectively) vs. the DNA and deduced AA sequences (GenBank accession number EF565131) found in the present work (third and fourth sequence, respectively).

accessed July 5, 2007). At first calving, average lactation milk production is about 262 kg in 150 d, and the content of milk fat is about 6.4%.

A 483-bp fragment containing exon 4 of the yak κ -casein gene was amplified by PCR. The primers used in PCR reactions were F: 5'-CGCTGTGAGAAAGAT-

GAAAG-3' and R: 5'-TCAGACCGCAGTTGAAGTAA-3' based on the sequence of the yak κ -casein gene exon 4 at GenBank (accession number AF194989). The PCR reaction was performed in a 25- μ L final volume containing 0.625 U of *Taq* DNA polymerase (TaKaRa, Dalian, China), 1 $\times$ PCR buffer (TaKaRa), 1.5 mM MgCl₂,

Table 1. Differences among domestic yaks in the κ -casein gene exon 4 nucleotide and corresponding AA (in parentheses)¹

GenBank acc. no.	Nucleotide position				GenBank submitter	Date of submission
	80	362	Between 427 and 428	482 ²		
AF194989	T (Leu)	G (Arg)	Absence	G	Fan et al.	October 14, 1999
AF030326	NA ³	C (Pro)	Presence	A	Ward et al.	October 18, 1997
AF030327	NA	C (Pro)	Presence	A	Ward et al.	October 18, 1997
AY095311	C (Pro)	C (Pro)	Presence	A	Prinzenberg et al.	April 11, 2002
AY095312	C (Pro)	C (Pro)	Absence	G	Prinzenberg et al.	April 11, 2002
EF565131	T (Leu)	C (Pro)	Absence	G	Present study	April 18, 2007

¹Nucleotide positions are compared with GenBank accession number AF194989.

²Indicates the second position of stop codon.

³NA indicates the nucleotide is not available from the sequence; absence and presence indicate a deletion and insert of 12 bp (sequence: CTT CTC CAG AAG, deduced AA: Leu Leu Gln Lys) between position 427 and 428, respectively.

200 μ M each dNTP, 0.4 μ M each primer, and approximately 100 ng of yak genomic DNA. Thermal cycling conditions were 95°C for 5 min; 30 $\times$ (94°C for 1 min, 54°C for 50 s, 72°C for 2 min); 72°C for 7 min.

The single-strand conformation polymorphism (SSCP) analysis was carried out as follows: 6 μ L of PCR product was added to 8 μ L of denaturing solution (0.05% of xylene-cyanole, 0.05% of bromophenol blue, and 0.02 M EDTA in deionized formamide). After heat denaturation (95°C for 10 min), the samples were immediately chilled on ice and then run for 10 h (150 V, 4°C) on 10% acrylamide:bisacrylamide gels (29:1) with 1% glycerol in 0.5 $\times$ Tris-borate EDTA buffer. Gels were silver stained according to the method of Bassam et al. (1991).

The PCR products showing different patterns on SSCP gels were cloned into the PCR II T-vector (Invitrogen, Carlsbad, CA) and transformed into competent cells of *Escherichia coli* XL₁-Blue (TaKaRa). The DNA was prepared and sequenced by using an ABI PRISM 377 DNA sequencer (Perkin-Elmer Cetus Instruments, Norwalk, CT). Eight samples were sequenced. Primers used for sequencing were the same as those used for the PCR-SSCP techniques. Sequence alignments, translations, and comparisons were carried out with Bioedit (Hall, 1999).

The PCR-SSCP analysis of yak κ -casein gene exon 4 revealed 2 distinct patterns (Figure 1). The more common pattern corresponded to the sequencing results of the GenBank accession number AF194989 (Fan et al., 1999; direct submission). The sequencing of the second pattern entered into GenBank with accession number EF565131 revealed a transversion G $\rightarrow$ C at position 362 of the referring sequence (GenBank accession number AF194989), resulting in the deduced AA exchange Arg₁₂₁ to Pro₁₂₁ of the mature protein sequence (Figure 2), and in 2 alleles named G and C, respectively, on the

basis of nucleotide 362. According to the restriction site of *Hind*III, this polymorphism does not fit with that described by Fan et al. (2000).

Theoretically, the AA exchange modifying the protein isoelectric point should be identifiable at the protein level by screening protein techniques, such as milk isoelectrofocusing, because Arg is basic and Pro is neutral. On the other hand, this AA substitution could also modify the biochemical micelle structure with important influences on milk quality and technological properties. Therefore, an investigation of yak κ -casein variability at the protein level is recommended for a more complete picture of the genetic polymorphisms in the κ -casein gene, and further investigations are needed to evaluate the influence of the mutation on quality and properties of yak milk.

In the Tianzhu White breed, the frequencies of genotypes for GG (n = 58), GC (n = 11), and CC (n = 6) were 0.77, 0.15, and 0.08, respectively. Therefore, the allele frequencies for G and C were 0.85 and 0.15, respectively. However, the frequencies of genotype for GG in the other breeds (Jiulong, Maiwa, and Datong) reached 1.00. Thus, allele C appears to be absent in the yaks from 3 breeds, suggesting the exclusive presence of this variant in the Tianzhu White breed. It should be noted that other previously described variants in yak are "C" at this point (Table 1) but they do not have Leu at position 27 of the mature protein.

The result of a test for Hardy-Weinberg equilibrium showed that the genotype distribution observed in the κ -casein gene did not correspond with that expected in the Tianzhu White breed ($P < 0.001$). The homozygous genotypes, in particular CC, are in excess, but the reasons for the discrepancies are not known. Therefore, more studies need to be done to understand this polymorphism, and to take into account the genetic polymorphism at the other casein genes in Tianzhu White

breed. Moreover, the absence of the C allele in the Jilong, Maiwa, and Datong breeds should be confirmed by increasing the numbers of typed animals.

To date, 5 different nucleotide sequences on exon 4 of the yak κ -casein gene are available at GenBank (accession numbers AF030326, AF030327, AF194989, AY095311, and AY095312). Among these 5 sequences, 4 polymorphic sites were observed in the 483-bp region amplified in the present work (nucleotides of position 1 to 126 are not available from AF030326 and AF030327 based on the nucleotide positions of AF194989). The nucleotide and corresponding AA differences among domestic yaks, including the new sequence EF565131 from the present work, are summarized in Table 1.

As observed from Table 1, and taking into account the new sequence EF565131 from the present work, no additional polymorphic site was observed among these sequences. Therefore, the polymorphism observed in the present work was already included in GenBank, but not previously described in yak populations. To our knowledge, this also is the first report on the polymorphism in Chinese yaks. The polymorphisms at positions other than 362 were not observed in exon 4 of the yak κ -casein gene in the present work.

The PCR-SSCP protocol developed in this study is a rapid method to analyze exon 4 of yak κ -casein gene. Further studies are evaluating the influence of the polymorphism described in the present study on properties of yak milk.

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