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Kappa Casein Gene Polymorphism in Holstein Chinese Cattle

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ABSTRACT

Kappa casein gene polymorphism has received a considerable attention because of its correlation with milk quality, composition and technological properties. The polymorphism of kappa casein gene (K-CN) was detected in Holstein Chinese cattle. A 218 bp sequence in exon IV of 319 Holstein Chinese cattle blood samples were amplified using polymerase chain reaction-single strand conformation (PCR-SSCP) technique. Sequence analysis revealed one single nucleotide polymorphism (SNP) T/C SNP in exon 1V at nucleotides (80), moreover; three genotypes TT, TC and CC were also identified with following frequencies: 0.40, 0.34 and 0.26%, respectively. The allele frequency for T and C found to be 0.6 and 0.4 %, respectively. Allele frequencies in the population fitted with Hardy–Weinberg equilibrium (P>0.05). Analysis of genetic polymorphism of k-casein at exon 1V exhibited medium polymorphism information content (PIC=0.36).

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INTRODUCTION

Studies on milk proteins are in progress for more than 100 years, but investigation of milk protein started more than 40 years ago and is still arousing research interest because of their crucial role in milk quality, composition and technological characteristics (Martin *et al*., 1999). The bovine milk specific proteins include casein fractions; α-1, α2, β and kappa–caseins (insoluble fractions), α-lactalbumin and β-lactogloulin which classified as (soluble fraction), (Galila and Darwish, 2008). Bovine kappa casein (K-CN) particularly, differs from other casein groups in structure and other characteristics. (K-CN) primary structure (169 residues) was determined by Mercier *et al.* (1973). The total size of kappa casein gene is about 13 KD divided into 5 exons and presents in several genetic variants in different cattle species but A and B were the most common variants. They are also associated with processing properties like cheese production technology (Alipanah *et al.*, 2007) and in physiological process such as cytotoxic and antibacterial effects that enhance the immunity (Botstein *et al.*, 1980; Matin and Otani, 2002). However, the other genetic variance has been reported in a few breeds but often at low frequencies (Sulimova *et al*., 2007). The identification of milk protein genotypes in dairy cattle provides a unique role of molecular genetics in the study of quantitative traits (Medrano and Aguilar, 1990).

Casein genetic polymorphisms are important and well documented due to their impact on quantitative and technological properties of milk. The association of genetic polymorphism with milk production and composition has stimulated interest in using genetic polymorphism of casein genes in molecular marker assisted selection (MAS) to improve milk performance traits in farm animals (Kumar *et al.*, 2006). Selection of k-casein alleles is a part of cattle breeding programs in many developed countries (Pedersen, 1991). Since, single nucleotide polymorphisms (SNPs) represent a potential resource for analyzing DNA sequence variation in many species of animals (Sukla *et al*., 2006). The aim of the present study was to detect possible polymorphisms of k-casein gene in Chinese Holstein cattle using PCR-SSCP technique.

MATERIALS AND METHODS

Samples collection and DNA extraction

A total of 319 Chinese Holstein cattle blood samples were collected from the experimental farm of Yangzhou University. The samples were taken from each cow from the jugular vein in a 10 ml vacuum tube containing acid citrate dextrose and stored in deep freezer at −20°C pending to DNA extraction. Genomic DNA was extracted using proteinase K digestion followed by standard phenol-chloroform extraction protocol (Mullenbach *et al*., 1989).

The quantity and quality of DNA were measured by spectrophotometer at 260/280 nm using an Eppendorf BioPhotometer (Germany). The content of DNA was estimated by ultraviolet spectrophotometer (Germany), and the genomic DNA was diluted to 50ng/µL.

PCR amplification

A 218 bp fragment containing exon 1V of kappa casein gene was amplified by PCR using forward 5′CTAAATCTGGCATAAAAGTA′3 and reverse 5′AATCACGGACTAAATAA′3, primers with accession No AY380228 sequence from gene bank. PCR was carried into 20µL final volume containing 100 ng template, 1µL 8 pmol/µL each primer, 0.4µL 10 mmol/µL dNTP, 1.0～2.4µL 25 mmol $MgCl_2$, 0.3µL 5 U Taq DNA polymerase and 2µL 10×buffer. PCR amplification reactions were used as follows: 94°C for 5 min (initial denaturation), followed by 30 cycles of (denaturation) 94°c for 1 min then (annealing) 50.6°c for 1 min and (extension) at 72°C for 1 min, and (final extension) at 72°C for 10 min. DNA implication was verified by electrophoresis of the PCR product with loading dye (95% formide, 0.25% bromophenol blue and 0.25% xylene cyanol) on 1.5% (W/V) agarose gel in 1X TAE, using DNA marker to confirm the desired PCR products length.

Single strand conformation polymorphisms

Single strand conformation polymorphism (SSCP) analysis said to be one of the most accurate and reliable technique for the identification of structural gene polymorphism that occur as a result of point mutation (Neibergs *et al.,* 1993; Barroso *et al.,* 1999). A total of 2.0 µL PCR product was mixed with 8 µL of the denaturation solution (50 mmol/L NaOH, 1 mmol/L EDTA), and 1 µL of the loading buffer containing 0.25% bromophenol blue and 0.25% xylene cyannole, denatured for 10 min at 98°C, and rapidly chilled at -20°C. The samples were then electrophoresed in 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A thermostatically controlled refrigerated circulator was used to maintain constant temperature (4°C) of the gels. The gels were run in the following conditions: 250 V, 40 mA, 10 min and 150 V, 24 mA, for 8 h. The gel was then silver stained. The patterns of DNA bands were observed and photographed with the GDS7500 System (UVP). Amplified PCR products of the different bands were directly sequenced by Shanghai Sangon Biological Engineering Technology & Services CO, Ltd, Shanghai, China.

Genotyping and allele's calculation

Genotypic and allelic frequencies for k-casein polymorphism in Chinese Holstein cattle were calculated using POPGENE software (ver.1.31).

Polymorphism information content

Polymorphism information content (PIC) was calculated according to the following equation as described by Botstein *et al.* (1980):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\left[\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2 p_j^2\right]$$

Where p_i is the frequency of the i^{th} allele, and n is the number of alleles.

RESULTS AND DISCUSSION

Polymerase chain reaction single strand conformation (PCR-SSCP) allows for the simultaneous typing of several alleles at casein loci, as well as the detection of unknown polymorphisms. (SSCP) analysis in the present work (Fig. 1 and 2) revealed one single nucleotide polymorphism (SNP). The polymorphic site consists of a single nucleotide substitution T/C at position 80 of the exon 1V. Sequence analysis (Fig. 2) also identified three patterns described as TT, TC and CC

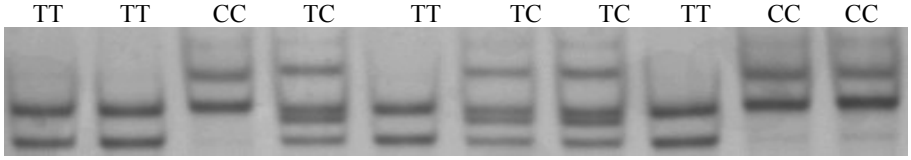

Fig. 1: Banding patterns determined by PCR-SSCP technique for the gene of kappa-casein in Chinese Holstein cattle

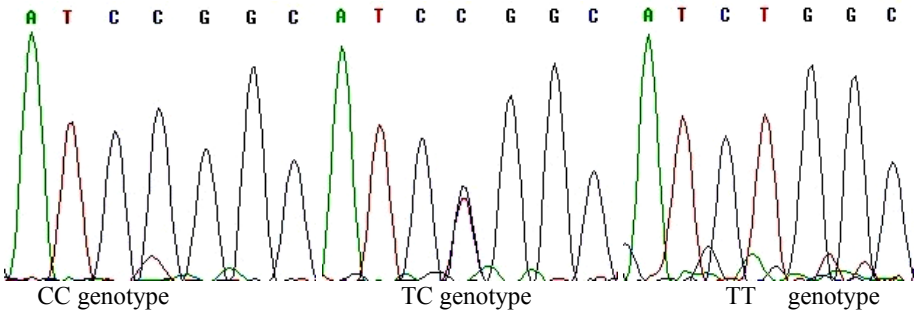

Fig. 2: Sequence patterns and genotypes of k-casein gene at exon 5 in Chinese Holstein cattle

Table 1: Genotype and allele frequency of k-casein gene exon 5

Genotype	No	Genotypic Frequency (%)	Allele	Allelic Frequency (%)	X^2	PIC
TT	128	0.40	T	0.6	15.01	0.36
TC	109	0.34	C	0.4		
CC	82	0.26				

genotype. Table 1 shows the genotypic and allelic frequency at kappa casein locus in Chinese Holstein cattle. They were 0.40, 0.34 and 0.26%, respectively; and 0.6 and 0.4% for allele T and C, respectively. Majority of studies in kappa casein genotyping and allele frequency have been considered only A and B variants; however, no other studies on Kappa casein alleles (T and C) were found in the literature. Genotype distribution for the studied population fitted with Hardy–Weinberg equilibrium (P>0.05).

The result of a test for Hardy-Weinberg equilibrium in the present study revealed that genotype distribution identified in kappa casein gene correspond with that demonstrated by Ma *et al*. (2007) and Ju *et al.* (2008) in southern Chinese Holstein cattle (P>0.05). Polymorphism within selected candidate genes is well documented for their impacts on quantitative traits for better understand for their influence and crucial role in marker-assisted selection (MAS) programs (Wu *et al*., 2005). Exon 1V of kappa-casein gene is very important as it contains most of the sequence coding for its molecule (Dogru and Ozdemir, 2009). The result of this study showed that this exon is polymorphic, where a SNP was detected at nucleotide (80). These results confirmed the findings stated by Ma *et al*. (2007), who demonstrated that exon 1V of kappa casein gene in Chinese Holstein cattle is polymorphic and they reported a T/C substitution.

Analysis of genetic polymorphism of k-casein at exon 1V exhibited medium polymorphism (PIC=0.36). The PIC value is commonly used in genetics as a measure of polymorphism for a marker locus used in linkage analysis, and also indicated the level of variability detected within a breed compared with the other breeds. PIC values ranged from 0.39 to 0.51 with an average of 0.44 (Buchanan and Thue, 1998), however, in the present study, a medium polymorphism was obtained (PIC=0.36). The PIC value obtained in this work is not similar to those reported by Buchanan and Thue (1998) who obtained PIC values ranged between 0.65 to 0.90 and also dissimilarity with those reported by Bishop *et al*. (1994), Kappes *et al.* (1997) and Viitala *et al.* (2003) who reported 0.68 PIC value for casein haplotype in Finnish Ayrshire dairy cattle. However, the present result agreed with those recorded by Ma *et al*. (2007), who analyzed the genetic polymorphisms of exon 4 and 5 at kappa casein gene in Southern Chinese Holstein Cattle by PCR-RFLP technique and obtained a moderate polymorphism (PIC=0.3511). This contradiction can be attributed to the fact that (PIC) for each bovine microsatellite loci varied quite considerably within the cattle breed as well (Buchanan and Thue, 1998).

This study confirms the results drawn by Ma *et al.* (2007) concerning the analysis of polymorphism of kappa casein gene at exon 1V in southern Chinese Holstein Cattle and it is possible to claim that this exon could be useful as genetic marker for additional improvements for this dairy breed.

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