



Kappa Casein Gene Polymorphism in Holstein Chinese Cattle

A. E. Hamza^{1*}, X. L. Wang and Z. P. Yang

Animal Science and Technology College, Yangzhou University, Yangzhou, China; ¹Department of Animal Production, College of Veterinary Science, Nyala University, Nyala-Sudan.

*Corresponding author: hamzab2007@hotmail.com; hamzab2003@yahoo.com

ARTICLE HISTORY

Received: May 24, 2010

Revised: June 17, 2010

Accepted: June 19, 2010

Key words:

Holstein Chinese cattle

Kappa casein gene

Polymorphism

PCR-SSCP

Single nucleotide

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 IV 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 IV exhibited medium polymorphism information content (PIC=0.36).

©2010 PVJ. All rights reserved

To cite this article: Hamza AE, XL Wang and ZP Yang, 2010. Kappa casein gene polymorphism in Holstein Chinese cattle. Pak Vet J, 30(x): xxx.

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, α 2, β and kappa-caseins (insoluble fractions), α -lactalbumin and β -lactoglobulin 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 50 ng/ μ L.

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 20 μ L final volume containing 100 ng template, 1 μ L 8 pmol/ μ L each primer, 0.4 μ L 10 mmol/ μ L dNTP, 1.0 μ L 25 mmol MgCl₂, 0.3 μ L 5 U Taq DNA polymerase and 2 μ L 10 \times buffer. PCR amplification reactions were used as follows: 94 $^{\circ}$ C for 5 min (initial denaturation), followed by 30 cycles of (denaturation) 94 $^{\circ}$ C for 1 min then (annealing) 50.6 $^{\circ}$ C for 1 min and (extension) at 72 $^{\circ}$ C for 1 min, and (final extension) at 72 $^{\circ}$ 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 (Neiberger *et al.*, 1993; Barroso *et al.*, 1999). A total of 2.0 μ L PCR product was mixed with 8 μ L of the denaturation solution (50 mmol/L NaOH, 1 mmol/L EDTA), and 1 μ L of the loading buffer containing 0.25% bromophenol blue and 0.25% xylene cyannole, denatured for 10 min at 98 $^{\circ}$ C, and rapidly chilled at -20 $^{\circ}$ 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 $^{\circ}$ 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

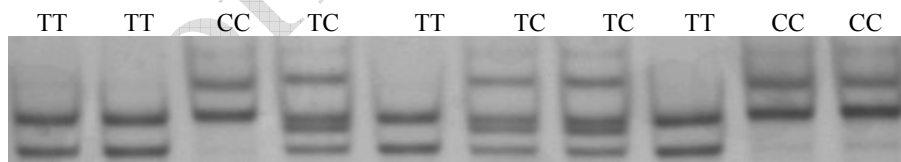


Fig. 1: Banding patterns determined by PCR-SSCP technique for the gene of kappa-casein in Chinese Holstein cattle

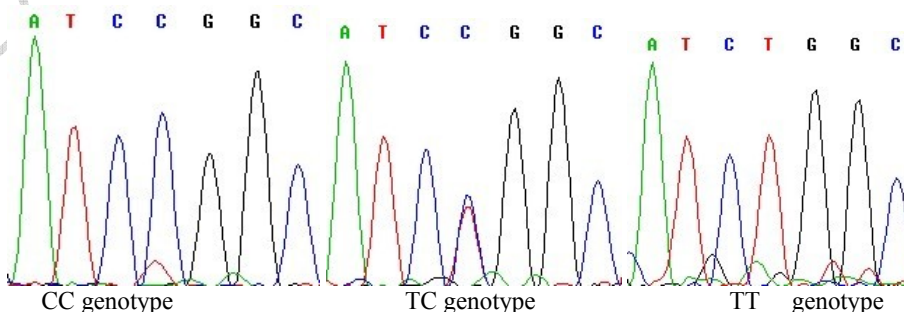


Fig. 2: Sequence patterns and genotypes of k-casein gene at exon 5 in Chinese Holstein cattle

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.

Acknowledgement

The authors are grateful to Dr. Hassan Hussein, Mr. Chinrinjin and Professor Yang's students for their appreciable help. The research was financially supported by the National "863" Program of China (No. 2008AA10Z144) and the Natural Science Foundation Research of University of Jiangsu Province (09KJA230002 and 08KJB230005).

REFERENCES

- Alipanah M, L Klashnikova and G Rodionov, 2007. K-casein genotypic frequencies in Russian breed Black and Red Pied cattle. *Iran J Biotechnol*, 3: 191-194.
- Barroso A, S Dunner and J Canon, 1999. A multiplex PCR-SSCP test to genotype bovine beta-casein alleles A1, A2, A3, B, and C. *Anim Genet*, 30: 322-323.
- Bishop MD, SM Kappes, JW Keele, RT Stone, SLF Sunden, GA Hawkins, S Solinas, R Fries, MD Grosz, J Yoo, and CW Beattie, 1994. A genetic linkage map for cattle. *Genetics*, 136: 619-639.
- Botstein D, RL White, M Skolnick and RW Davis, 1980. Construction of a genetic linkage map in man using restriction fragment polymorphisms. *Am J Hum Genet*, 32: 314-331.
- Buchanan FC and TD Thue, 1998. Inbred polymorphic information content of microsatellites in cattle and sheep. *Can J Anim Sci*, 78: 425-428.
- Dogru U and M Ozdemir, 2009. Genotyping of kappa-casein locus by PCR-RFLP in brown Swiss cattle breed. *J Anim Vet Adv*, 8: 779-781.
- Galila ASE and SF Darwish, 2008. A PCR-RFLP assay to detect genetic variants of kappa-casein in cattle and buffalo. *Arab J Biotech*, 11: 11-18.
- Ju Z, Q Li, H Wang, J Li, O An, W Yang, J Zhong and F Wang, 2008. Genetic polymorphism of kappa-casein gene exon4 and its correlation with milk production traits in Chinese Holsteins. *Heredity*, 10: 1312-1318.
- Kappes SM, JW Keele, RT Stone, RA McGraw, TS Sonstegard, TPL Smith, NL Lopez-Corrales, and CW Beattie, 1997. A second-generation linkage map of the bovine genome. *Genet Res*, 7: 235-249.
- Kumar D, N Gupta, S Ahlawat, R Satyanarayana, S Sunder and S Gupta, 2006. Single strand confirmation polymorphism (SSCP) detection in exon I of the lactalbumin gene of Indian Jamunapri milk goats (*Capra hircus*). *Genetic Mol Biol*, 29: 271-274.
- Ma X, X Wang, G Hu, G Ma, J Zhao, C Peng and G Chang, 2007. Analysis of genetic polymorphisms at kappa-CN Exon 4 and Exon 5 in southern Chinese Holstein Cattle. *China Dairy Cattle*, 2: 5-8.
- Martin P, M Ollivier-Bousquet and F Grosclaude, 1999. Genetic polymorphism of caseins: a tool to investigate casein micelle organization. *Int Dairy J*, 9: 163-171.

- Matin MA and H Otani, 2002. Cytotoxic and antibacterial activities of chemically synthesized κ -caseicin and its partial peptide fragments. *J. Dairy Res*, 69: 329-334.
- Medrano JF and EA Cordova, 1990. Genotyping of bovine κ -casein loci following DNA sequence amplification. *Biotechnology*, 8: 144-146.
- Mercier J, G Brignon and B Rebaeau-Dumas, 1973. Structure primaire de la caseine κ^B bovine. Sequence complete. *Eur J Biochem*, 115: 113-122.
- Mullenbach R, PJ Lagoda and C Welter, 1989. An efficient salt chloroform extraction of DNA from blood and tissues. *Trends Genet*, 5: 391.
- Neibergs HL, AB Dietz and JE Womack, 1993. Single strand conformation polymorphisms (SSCPs) detected in five bovine genes. *Anim Genet*, 24: 81-84.
- Pedersen J, 1991. Selection to increase frequency of kappa-casein variant B in dairy cattle. *J Anim Breed Genet*, 108: 434-445.
- Sukla S, TK Bhattacharya, RT Venkatachalapathy, P Kumar and A Sharma, 2006. Cloning and characterization of alpha (s2)-casein gene of Riverine buffalo. *DNA Seq*, 17: 458-464.
- Sulimova GE, MA Ahani, J Rostamzadeh, MMR Abadi and OE Lazibny, 2007. K-casein gene (CSN3) allelic polymorphism in Russian cattle breeds and its information value as a genetic marker. *Russian J Genet*, 43: 88-95.
- Viitala SM, NF Schulman, DJ de Koning, K Elo, R Kinoshita, A Virta, J Virta, A Ma'ki-Tanila, and JH Vilkki, 2003. Quantitative trait loci affecting milk production traits in Finnish Ayrshire dairy cattle. *J. Dairy Sci*, 86: 1828-1836.
- Wu XL, DM Michael, De Sachinadan, QJ Xiao, JJ Michel, CT Gaskins, JJ Reeves, JR Busboom, RW Wright Jr and Z Jiang, 2005. Evaluation of candidate gene effects for beef back fat via Bayesian model selection. *Genetica*, 125: 103-113.

Uncorrected Proof