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Genetic polymorphism of kappa-casein gene in indigenous Eastern Africa goat populations

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Indigenous goat breeds kept by majority of smallholder rural farmers in Eastern Africa are adapted to the local environment. These goats are critical for nutrition and income of their keepers. Milk production per doe is extremely variable. The variation in milk yield in goats is due to varied management practices and variability in genetic make-up of the animals. The variation in kappa-casein gene and the distribution frequencies of its variants amongst indigenous Eastern Africa goat populations were investigated. A 458 base pairs sequence in exon 4 of 296 goat samples were amplified, sequenced and variation analyzed. Nine point mutations corresponding to base transitions were identified. Three sites were synonymous substitutions while the other six mutations were non-synonymous. All the amino acid substitutions were conservative. Analysis of the association of the mutations yielded nine haplotypes. The occurrence of these haplotypes in ten goat populations indicated that only one haplotype occurred at a rather high frequency. The prevalent κ -casein variant was CSN3*B with frequencies ranging from 0.750 to 0.953. The second most common allele was CSN3*A. Further studies on other casein loci are necessary to establish associations of all the casein mutations and the effects of the haplotypes to milk production traits.

Key words: Goat, indigenous, k-casein, polymorphism.

INTRODUCTION

Indigenous goats are naturally highly adapted to hot environments and can withstand recurrent drought in Eastern Africa better than cattle. Goats are important to the subsistence, economic and social livelihoods of a large human population in the region. They are especially important to women, children and the aged who are often the vulnerable members of the society in terms of under-nutrition and poverty (Kosgey, 2004). Goat milk exceeds cow milk in monounsaturated, polyunsaturated fatty acids and medium chain triglycerides in which all are known to be beneficial for human health, especially for cardiovascular conditions (Haenlein, 1992). However, milk pro-

duction per doe is relatively low and extremely variable. The variation in milk yield being due to management practices like disease control, feeding and housing and variability in genetic make-up of the goats (Azevedo et al., 1994). This provides an opportunity for improvement of goat milk production by changes in management practices and adoption of genetic improvement technologies.

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 productivity in farm animals (Kumar et al., 2006). Casein is made up of many components; the main ones are α_{s1} -casein, α_{s2} -casein, β -casein and κ -casein (Walstra, 1999). Kappa-casein plays an important role in the formation, stabilization and aggregation of the casein

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micelles thus altering the manufacturing properties and digestibility of milk (Jann, 2004). The casein proteins are encoded in a locus that comprises four casein genes; the evolutionary related calcium - sensitive casein encoding genes (α_{s1} , α_{s2} and β) and the functionally related κ -casein gene (Rijnkels et al., 1997). Kappa-casein gene (CSN3) sequence and the promoter region of the gene have been reported (Coll et al., 1993, Coll et al., 1995). The goat κ -casein mRNA contains an open reading frame of 579 bp coding for 21 amino acids for signal peptide and 171 amino acids of mature protein, the coding sequence for mature protein is contained in exon three (9 amino acids) and exon four (Yahyaoui et al., 2003).

Studies that have examined the CSN3 in cattle have indicated an association of some κ -casein alleles and milk yield, composition and quality. Studies by Bovenhuis et al. (1992) which involved 305 days milk production records of 10,151 first lactation cows observed that κ -casein genotypes had a significant effect on milk production. Kappa-Casein AA cows produced 173 kg of milk more than κ -casein BB cows. Furthermore, κ -casein genotypes had a highly significant effect on protein content; the κ -casein BB cows produced milk with a 0.8% higher protein content than that of the AA cows. Generally, majority of researchers believe that the κ -casein B variant is associated with higher fat, protein and casein in the milk and has a significant influence on cheese making properties of milk and superior rennet coagulation properties in comparison to AA or AB variants (Gangaraj et al., 2008). The genotypes BB and AB are used in artificial insemination programs to obtain a greater increase of the frequency of these alleles in cattle populations of commercial interest (Otaviano et al., 2005).

Caseins are rapidly evolving gene family, presumably due to the minimal structural requirements for functioning (Bonsing and Mackinlay, 1987). Whereas CSN3 is considered to be monomorphic in sheep (Moioli et al., 1998), however, recent studies on goat CSN3 showed that the gene is highly polymorphic (Caroli et al., 2001; Yahyaoui et al., 2001; Angiolillo et al., 2002; Yahyaoui et al., 2003; Chessa et al., 2003; Jann et al., 2004; Reale et al., 2005; Prinzenberg et al., 2005). A total of 16 polymorphic sites have been identified in the domesticated goat, of which 13 are protein variants and 3 are silent mutations involving a total of 15 polymorphic sites in CSN3 exon 4 (Prinzenberg et al., 2005). Recent studies emphasised, that the analyzed breeds show differences in the occurrence and frequency of the alleles, the allele distribution reflecting the geographic origin (Moioli, 2007). The distribution of such alleles has been influenced either by selection pressure for milk production or, more likely, by genetic drift (Yahyaoui et al., 2003).

The purpose of the current study was to analyze the genetic polymorphism of κ -casein gene exon 4 by sequencing. And further determine the distribution frequencies of the variants in one exotic and nine indigenous goat populations found in five countries in Eastern Africa. This study

serves as the bases for the design of an association studies that will provide information on breeding of goats that have better milk production traits.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of nine indigenous goat populations from five Eastern Africa countries were considered for the study. In addition, one exotic breed was also analysed. Small East Africa goat (n = 35) Kenya (Baringo district), Small East Africa goat (n = 27) Kenya (Samburu district), Long-Eared Somali (n = 27) Ethiopia, Maasai (n = 30) Tanzania, Keffa (n = 32) Ethiopia, Afar (n = 33) Ethiopia, Shilluk (n = 16) Sudan, Short-Eared Somali (n = 35) Somalia, Long-Eared Somali (n = 28) Somalia, Toggenberg (n = 33) Germany.

Genomic DNA was isolated from the whole blood samples using the method described by Sambrook et al. (1989) with minor modifications. After checking the quantity and quality of the DNA using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE), the DNA was diluted to a final concentration of 50 ng μ l⁻¹ in water and stored at 4°C, until the time for use.

DNA amplification and purification

A 458 bp fragment of goat κ -casein exon 4 was amplified by polymerase chain reaction (PCR) from gDNA (genomic DNA) samples using primers CSN3_1A (TATGTGCTGAGTAGGTATCC) and CNS3_1B (TTGTCCCTTTTGTGATGTCTCC) which were designed relative to the caprine κ -casein cDNA (Coll et al., 1993). The PCR was performed in a 15 μ l final volume containing primer CNS3_1A (20 pM; 0.15 μ l), primer CNS3_1 B (20 pM; 0.15 μ l), ABgene Reddy mix (7.5 μ l), water (5.7 μ l) and gDNA (1.5). Thermal cycling conditions was: 94°C for 4 min, 5 cycles of 94°C for 15 s, 59°C for 1 min and 70°C for 30 s, followed by 30 cycles of 94°C for 15 s, 54°C for 30 s, 70°C for 30 s, with a final extension at 72°C for 10 s. To confirm amplification, PCR products were resolved by agarose gel electrophoresis and visualized by gel red staining. PCR products were purified using the QIAquick PCR purification kit (QIAGEN GmbH, Germany).

DNA sequencing reactions

Purified fragments were sequenced to determine the precise nucleotide sequence. Direct sequencing of CSN3 exon 4 was performed using the same primers used for amplification that is primers CSN3_1A and CSN3_1B. Sequencing was done using the BigDye® Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems). The purified products from the BigDye® Terminator were electrophoresed on an ABI 3730 XL automated capillary DNA sequencer (Applied Biosystems) for one hour and the resulting electropherograms were analyzed.

Sequence data analysis

BioEdit (Ibis Biosciences) was used for the viewing and editing of the DNA sequences. Multiple alignments of the sequences were performed using clustalX program (Thompson et al., 1997). The polymorphic sites were confirmed by visual examination of the electropherograms. The translation of DNA sequence to amino acid sequence was done using ExPASy - Translate tool. Haplotypes were inferred using Clark's algorithm (Clark, 1990) and their fre-

Table 1. Kappa-casein gene haplotypes among indigenous Eastern Africa goats.

Nucleotide position	Protein position	Haplotypes								
		A	B	D	L	M	N	O	P	Q
245	43	T (Tyr)		C (Tyr)		C (Tyr)	C (Tyr)			C (Tyr)
247	44	A (Gln)		G (Arg)						
309	65	G (Val)			A (Ile)					A (Ile)
384	90	G (Asp)				A (Asn)	A (Asn)			
471	119	G (Val)	A (Ile)	A (Ile)	A (Ile)	A (Ile)	A (Ile)		A (Ile)	
545	143	C (Asn)						T (Asn)		
550	145	T (Val)				C (Ala)	C (Ala)			
584	156	G (Ala)					A (Ala)		A (Ala)	
591	159	T (Ser)		C (Pro)	C (Pro)		C (Pro)			C(Pro)

Nucleotide positions are compared with GenBank accession No. X60763.

frequencies determined by direct counting.

RESULTS

Variation in κ -casein gene

CSN3 exon 4 sequences from 296 goats were analyzed for variations. Nine polymorphic sites were identified (Table 1). All were point mutations; corresponding to base transitions, where T and G are substituted by C and A, respectively. Most of the polymorphic sites were heterozygous, except at position 471 which was homozygous for genotype AA in 90% of the goats (266) and heterozygous for genotype AG in the rest.

Analysis of the deduced amino acid sequences showed that three sites were synonymous substitutions corresponding to amino acid residues tyrosine (at position 245), asparagine (545) and alanine (584). The other six mutations were non-synonymous producing codon changes of glutamine to arginine (at position 247), valine to isoleucine (309 and 471), aspartate to asparagine (384), valine to alanine (550) and serine to proline (591) (Table 1). All the amino acid substitutions are conservative.

Kappa-casein haplotypes

Nine haplotypes were inferred using Clark's algorithm from the 296 animals (592 chromosomes) sampled. Table 1 shows the haplotype alleles at the variable sites. The analysis of the κ -casein haplotypes in the ten goat populations indicated that only one haplotype occur at a rather high frequency. The prevalent κ -casein variant was CSN3*B with frequencies ranging from 0.750 (Shilluk) to 0.953 (Keffa). The second most common allele was CSN3*A. This allele was inconsistent in that it was not found in three goat populations, while in the rest, the frequencies ranged from low value of 0.014 (Short Eared goat) to a relatively high value of 0.250 (Shilluk). The haplotype frequency in the populations under study is shown in Table 2.

DISCUSSION

Kappa-casein gene variation

K-casein gene exon 4 from 296 goats collected from ten populations, obtained from five Eastern African countries were analyzed for sequence variations. A total of nine SNPs were identified. Seven SNPs had been identified previously in other goat populations. Yahyaoui et al. (2001), first identified polymorphism in Spanish and French breeds in the positions 245 (nucleotide change from T to C), 309 (G to A), 471 (G to A) and in the position 591 (T to C). Polymorphism in the position 247 (A to G) was first identified by Caroli et al. (2001) in Italian goat breeds. While polymorphism in the positions 384 (G to A) and 550 (T to C) was previously identified by Prinzenberg et al. (2005). These SNPs have also been described in various goat populations outside Eastern Africa (Yahyaoui et al., 2003; Angiolillo et al., 2002; Jann et al., 2004). The high number of similarity in polymorphic sites amongst most of the populations in different parts of the world is suggestive of similarity in evolutionary processes undergone by these populations. Two new SNPs identified at nucleotide positions 545 (C/T) and 584 (G/A), occurred in two (Small East Africa goat-Baringo district and Maasai) and three (Long Eared Somali, Short Eared Somali- Somalia and Long Eared Somali-Somalia) goat populations, respectively. The two new SNPs are neutral mutations that are of no consequence on κ -casein protein structure and function. However, they can be used as markers if they are established to be in linkage disequilibrium with alleles associated with milk production traits. Most of the genotypes at polymorphic sites were heterozygous. However, polymorphic site 471 was homozygous for genotype AA in 90% of the samples analyzed, agreeing with earlier observation (Mercier et al., 1976) which postulated isoleucine to be the predominant amino acid at the corresponding amino acid sequence.

Three of the sites are silent mutations corresponding to

Table 2. Haplotype frequencies for the kappa-casein locus in ten goat populations analyzed.

Population	n.	A	B	D	L	M	N	O	P	Q
SEAgB	35	0.029	0.913	-	-	0.029	-	0.029	-	-
SEAgS	27	0.019	0.944	-	-	0.037	-	-	-	-
LES	27	0.037	0.926	-	-	0.019	-	-	0.019	-
MS	30	0.033	0.933	-	-	-	-	0.033	-	-
KF	32	-	0.953	-	0.016	-	-	-	-	0.031
AF	33	-	0.939	0.045	-	-	-	-	-	0.015
SH	16	0.250	0.750	-	-	-	-	-	-	-
TG	33	0.121	0.879	-	-	-	-	-	-	-
SES-S	35	0.014	0.914	-	0.029	-	0.014	-	0.014	0.014
LES-S	28	-	0.946	-	-	0.036	-	-	0.018	-

SEAgB, Small East Africa goat (Baringo district); Small East Africa goat (Samburu district); LES, Long Eared Somali; MS, Maasai; KF, Keffa; AF, Afar; SH, Shilluk; TG, Toggenberg; SES-S, Short Eared Somali-Somalia; LES-S, Long Eared Somali-Somalia. n., Sample size. Haplotype frequencies calculated using chromosome numbers (= 2n).

amino acid residues tyrosine (at position 245), asparagine (545) and alanine (584). The other six mutations were non-synonymous producing codon changes of glutamine to arginine (at position 247), valine to isoleucine (309 and 471), aspartate to asparagine (384), valine to alanine (550), serine to proline (591). Individual κ -casein molecules have been speculated to cross-link into disulfide-bonded polymers with a structure such that the hydrophilic tails project into the milk serum and the hydrophobic regions attach to the micelle core containing calcium-sensitive caseins (Phadungath, 2005). All the non-synonymous single nucleotide polymorphisms caused conservative amino acid substitutions. Thus, the mutations are of less consequence in the function of the κ -casein protein of stabilization of the micelles.

Kappa-casein haplotypes in ten goat populations

A total of nine haplotypes were inferred using Clarks' algorithm (Clark, 1990). Nomenclature developed by Prinzenberg et al. (2005) was adopted in naming previously identified haplotypes, while the new haplotypes were named in alphabetical order following the lastly named haplotype. Out of the nine haplotypes identified, five had previously been found in other goat breeds (Yahyaoui et al., 2001; Caroli et al., 2001; Angiolillo et al., 2002; Yahyaoui et al., 2003; Jann et al., 2004; Prinzenberg et al., 2005).

The analysis of κ -casein variation in ten goat populations indicated that only one haplotype occurred at a rather high frequency. The most prevalent CSN3 haplotype was CSN3*B with frequencies ranging from 0.750 (Shilluk) to 0.953 (Keffa). The haplotype is most likely to be associated with production trait(s) which has/have been selected for, by the local communities, over many generations. This haplotype appears to be almost fixed in these populations. The second most common allele was CSN3*A. This allele was inconsistent in that it was not

found in three goat populations (Keffa, Afar and Long Eared Somali-Somalia); this inconsistency might be due to low number of samples analyzed and low haplotype frequency in most of the populations. The frequencies in the rest of the populations ranged from low value of 0.014 (Short Eared Somali-Somalia) to a relatively high value of 0.250 (Shilluk). These findings were in agreement with earlier report by Prinzenberg et al. (2005) in goat breeds from Europe, Africa and Asian part of Turkey, their most common variant was also CSN3*B with frequencies ranging from 0.260 (Hair goat) to 0.674 (Angora goat). Their second most common allele was also CSN3*A, with frequencies ranging from 0.151 (Angora goat) to 0.414 (Borno goat). Two variants were found in only one population each. The haplotype CSN3*D was only found in Afar with a relatively low frequency of 0.045 while variant CSN3*N appeared in Short Eared goat population with a low frequency of 0.014. The alleles CSN3*L and CSN3*O occurred in low frequencies in two goat populations; CSN3*L was found in Short Eared Somali population (Somalia) with a relatively low frequency of 0.029 and in Keffa goat population with a rather very low frequency of 0.016. Whereas, allele CSN3*O appeared in Maasai population with a frequency of 0.033 and in Small East Africa goat (Baringo district) the frequency was 0.029. Haplotype CSN3*M was the most widely distributed variant occurring in four goat populations (Small East Africa goat-Baringo district, Small East Africa goat-Samburu district, Long eared Somali, and Long Eared Somali-Somalia). Based on the high frequency (approximately 90%) of haplotype CSN3*B in nine out of the ten goat populations, it appears that the populations are rather similar at this locus. The relatively low sample size of Shilluk goat population might be the cause of its slight variations in haplotype frequencies. However, due to independent calculation of haplotype frequencies in each population samples using chromosome numbers, the low sample size of Shilluk population does not affect the haplotype frequencies in other goat populations.

The greatest number of haplotypes at CSN3 (six) occurred in Short Eared Somali-Somalia, CSN3*B was at high frequency followed by CSN3*L, while the rest occurred at equal frequency: CSN3*A, CSN3*N, CSN3*P and CSN3*Q. Four alleles were found in Small East Africa goat (Baringo district) and Long Eared Somali. Shilluk and Toggenberg were almost monomorphic at this locus, with allele CSN3*A occurring at a low frequency of 0.250 and 0.121, respectively. In future, it is necessary to determine nucleotide variations within regions of the CSN3 not analyzed in this study, these includes the promoter region, exon 1, 2 and 3 and introns and variation in other casein loci.

Conclusion

CSN3 is a highly polymorphic locus with a total of seventeen SNPs identified in various goat populations globally as at now. Out of the total SNPs, only nine were identified in Eastern Africa goat populations, two of which had not been previously identified in other goat populations. Six out of the nine SNPs are non-synonymous mutations, leading to conservative amino acid substitutions. With the inclusion of the newly inferred haplotypes, the total number of κ -casein haplotypes have rapidly increased from sixteen to twenty. In Eastern Africa goat populations, nine haplotypes were inferred, four of which had never been identified in other goat populations. CSN3*B remain the most common haplotype amongst the majority of breeds in various geographical locations, including Eastern Africa goat populations. On considering the frequencies of the CSN3 haplotypes in the goat populations studied, it appears that variation in this gene may not influence milk yield; therefore, further studies on other casein loci are necessary to establish associations of all the casein mutations and the effects of such haplotypes on milk production traits.

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