DNA Polymorphisms of κ-Casein, β-Lactoglobulin, Growth Hormone and Prolactin Genes in Korean Cattle

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ABSTRACT : The gene and genotypic frequencies of κ-casein (κ-CN), β-lactoglobulin (β-LG), growth hormone (bGH) and prolactin (bPRL) loci in Korean cattle were investigated using PCR-RFLP analyses. Genomic DNA samples were obtained from 290 cows and 30 AI bulls. In both cows and bulls, the most predominant genotypes of κ-CN, β-LG, bGH and bPRL loci were AB, BB, AA and AA, respectively. The frequencies of A and B alleles for κ-CN locus were .612 and .388 for cows and .567 and .433 for bulls. The respective frequencies of A and B alleles for β-LG locus were .153 and .847 in cows and .217 and .783 in bulls. The frequencies of A and B alleles for bGH locus were .769 and .231 in cows and .784 and .216 in bulls, respectively. The frequencies of A and B alleles for bPRL locus were .678 and .322 for cows and .767 and .233 for bulls. Differences in frequencies of these alleles were not significant between cows and bulls at all loci examined. If the DNA polymorphisms of these candidate genes are associated with economically important traits, they could serve as genetic markers for genetic improvement in future marker-assisted selection programs in Korean cattle.
(**Key Words:** κ-CN, β-LG, bGH, bPRL, PCR-RFLP, Gene Frequency, Korean Cattle)

INTRODUCTION

Genetic polymorphisms are playing an increasingly important role as genetic markers in many fields of animal breeding. With the development of molecular genetic techniques, it had become possible to estabilish a new class of genetic markers based upon variability at the DNA sequence level. In particular, restriction fragment length polymorphisms (RFLPs) provide some new hope for the practical application of polymorphic genetic markers to livestock improvements (Soller and Beckmann, 1982). The discovery of RFLP generated renewed interest in the use of genetic marker loci as an aid to selection programs. If one (or several) of thses RFLP markers can be shown to be associated with quantitative trait loci (QTL) or economic trait loci (ETL), these can be used for selection criteria. This process of selection has become known as marker-assisted selection (MAS) or genotype-assisted selection (GAS). In a breeding scheme, use of phenotypic and marker data could provide more information than phenotype alone. The use of information on genetic markers is expected to accelerate genetic progress through increasing accuracy of selection, reduction of generation interval and increasing selection differentials (Soller and Beckmann, 1983; Kashi et al., 1990; Meuwissen and van Arendonk, 1992).

The milk protein and hormone genes are excellent candidate genes for linkage analysis with QTL because of their biological significance on the quantitative traits of interest (Moody et al., 1996). Milk protein genetic polymorphisms have received considerable research interests in recent years because of possible associations between milk protein genotypes and economically important traits in dairy cattle. Milk protein genes such as κ-casein (κ-CN) and β-lactoglobulin (β-LG) are associated with milk production performance and have a major influence on the composition of milk and on the processing properties of milk (Ng-Kwai-Hang et al., 1990; Bovenhuis et al., 1992; Cowan et al., 1992; Lin et al., 1992). Therefore, milk protein genes might be useful as genetic markers for the additional selection criteria in dairy cattle breeding. It is well known that bovine growth hormone (bGH) has a major impact on growth, lactation and mammary gland development in cattle. Several researchers also reported associations between RFLP marker of bGH and milk production traits in dairy cattle (Hoj et al., 1993; Lucy et al., 1993; Falaki et al., 1996;

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Lee et al., 1996). Rocha et al. (1992) found that GH gene RFLP was associated with beef cattle characteristics such as live weight, maternal trait and shoulder width of calves at birth. Prolactin (bPRL) plays an important regulatory function in differentiation of epithelial cells in mammary tissue and in milk secretion (Akers et al., 1981). Therefore, allelic variation in the structural or regulatory sequencies of these candidate genes would be of interest because of possible direct or indirect effects on milk production and growth performance (Falaki et al., 1996).

Until now most of the characterization of these candidate genes has been done in dairy cattle, whereas very little has been done in beef cattle. The possibilities for genetically improving the population for a single gene are determined in part by the frequencies of the alleles (Falconer, 1989). These candidate genetic variants differ from breed to breed in their occurrence and frequency. Thus, information is required on the allele frequencies of potential candidate genes in the cattle breed population. The Korean cattle are unique indigenous native cattle breed and play an important role as a beef producer and as a valuable animal genetic resource in Korea. The objective of this study was to analyze the DNA polymorphisms of κ-CN, β-LG, bGH and bPRL by using PCR-RFLP method and to determine the gene and genotype frequencies of these loci in Korean cattle.

MATERIALS AND METHODS

Animals

A total of 320 Korean cattle were used in this study. Blood samples were collected from 290 cows at the Daekwanryeong Branch, National Livestock Research Institute, R. D. A. Semen samples were also obtained from 30 AI bulls.

DNA genotyping

Genomic DNA was extracted from blood using the saturated salt extraction procedure by Miller et al. (1988) and from semen as described by Zadworny and Kuhnlein (1990).

Genotyping of κ-CN, β-LG, bGH and bPRL was from analysis of DNA using PCR-RFLP technique. The primer sequencies and restriction enzymes used in PCR-RFLP analysis are given in table 1. Briefly, for each gene a specific fragment was amplified using specific primers and PCR. Amplified products were digested with specific a restriction enzyme and then separated on 8-10% polyacrylamide gels for κ-CN and β-LG and 3% agarose gels for bGH and bPRL. Gels were then stained with ethidium bromide and photographed with UV transillumination. Procedures for amplifying DNA fragments, digestion of the amplified products and seperation of the resulting fragments have been discussed previously (Chung et al., 1994, 1995[b], 1996[a,b]).

Statistical analysis

Allele frequencies for each gene were determined by direct gene counting method and standard error of frequencies was calculated as $\sqrt{Pi\ (1-Pi)/2N}$, where N is the sample size and Pi is the frequency of the i-th allele at a locus. Allele frequencies between cows and bulls were compared using contigency table chi-square tests.

RESULTS AND DISCUSSION

The κ-CN, a protein containing 169 amino acids, has four genetic variants A, B, C and D (Eigel et al., 1984). Two of the these alleles, A and B, have been identified in Korean cattle (Chung et al., 1995[a]). The genetic differences between the A and B alleles are caused by two point mutations in the exon 4 of this gene (Stewart et al., 1984; Gorodetsky and Kaledin, 1987; Alexander et al., 1988). κ-CN A allele differs from B by having Ile (ATC) and Ala (GCT) at amino acid positions 136 and 148, respectively, instead of Thr (ACC) and Asp (GAT). The restriction fragment patterns characterizing the three κ-CN genotypes are shown in figure 1. An 874 bp fragment between nucleotides 10,592 and 11,466 from exon IV to

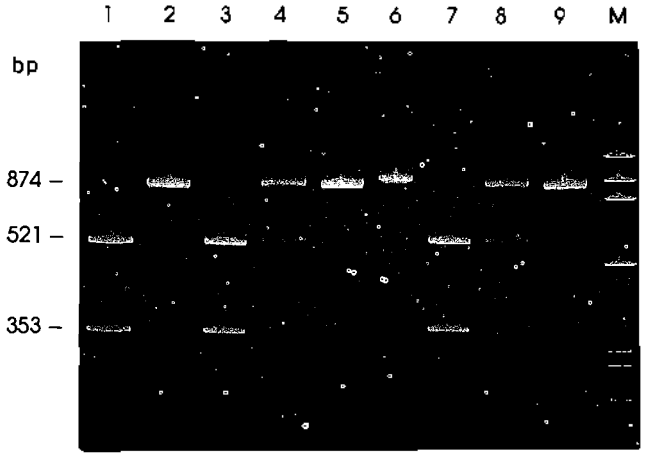

Figure 1. Restriction fragment length polymorphism of κ-CN gene in 8% polyacrylamide gel following digestion with Hind III enzyme of PCR products. In the gel, lane 6 represents the undigested PCR product(a 874bp fragment), lanes 2, 5 and 9 represent AA genotypes(874 bp band only), lanes 1, 3 and 7 represent BB genotypes(521 and 353 bp bands), lanes 4 and 8 represent AB genotypes (874, 521 and 353 bp bands) and lane M represents the molecular size marker (Φx-174 DNA / Hae III marker).

Table 2. Genotype and gene frequencies of κ-CN, β-LG, bGH and bPRL loci in Korean cattle population

Locus	Cows (n=290)					Bulls (n=30)				
	Genotype			Allele		Genotype			Allele	
	AA	AB	BB	A	B	AA	AB	BB	A	B
κ-CN	.386	.452	.162	.612±0.020[1]	.388±0.020	.300	.533	.167	.567±0.064	.433±0.064
β-LG	.052	.203	.745	.153±0.015	.847±0.015	.033	.367	.600	.217±0.053	.783±0.053
bGH	.558	.421	.021	.769±0.018	.231±0.018	.600	.367	.033	.784±0.053	.216±0.053
bPRL	.510	.335	.155	.678±0.019	.322±0.019	.633	.267	.100	.767±0.018	.233±0.018

[1] Standard error.

has been suggested that bPRL locus has different allele frequencies among the cattle breeds. Differences in allele frequencies indicate the possibility of differences in genetic backgrounds among cattle breed populations. Table 3 shows the contigency table chi-square test statistics that were calculated when testing for differences in allele frequencies between cows and bulls populations. Results indicate that no significant differences in allele frequencies exist between these two populations at all loci examined.

Comprehensive investigation of the relationships among genetic polymorphisms and phenotypic traits requires evaluation of allele frequencies for the various candidate genes known to have critical roles in the phenotypic traits of interest. If these alleles of candidate genes are associated with genetic potential, they could serve as informative markers and assist attempts to identify superior animals at an early age.

Table 3. Comparisons of allele frequency between cows and bulls using contigency table chi-square tests

Locus	χ^2	df	p
κ-CN	0.059	1	< .900
β-LG	1.622	1	< .250
bGH	0.065	1	< .900
bPRL	2.003	1	< .250

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