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Novel variations in 5′ flanking region of the α-Lactalbumin gene in exotic and indigenous cattle

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ABSTRACT

α-Lactalbumin (α-La) gene is involved in milk production traits in dairy animals. The variability in milk production potentials of different animals have been attributed to the polymorphism existing within this gene. The study was carried out to detect polymorphism in PCR amplified DNA fragments encompassing to − 363 to + 80 of the α-La gene in 50 Holstein Friesians (HF), 63 Jersey and 49 endogenous Kashmiri cattle. The polymorphism was detected by sequence analysis of amplified products. Three genotypes designated as AA, AB, BB were observed during the course of the study. The two alleles were differentiated on the basis of three single nucleotide polymorphism (SNP) each at position − 250, − 89, and − 46 of the promoter region of the α-La gene. All the three nucleotide variations involved base transition viz., A to G, T to C and A to G, respectively. The haplotype with nucleotide A, T and A at positions − 250, − 89 and − 46 was designated as A whereas the haplotype with nucleotide G, C and G at positions − 250, − 89 and − 46 was designated as allele B. The genotypic frequencies were observed to be 90.50% (n = 57) for AA allele and 9.50% (n = 06) for AB allele in Jersey, however no animal with genotype of BB allele was observed. The genotypic frequencies of homozygous A, homozygous B and heterozygous AB were 30% (n = 15), 22% (n = 11) and 47% (n = 23), respectively in Kashmiri cattle. Like in Jersey breed of cattle, no homozygous animal was observed to harbor BB genotype in HF cattle and the frequencies of AA and AB genotypes were 70% (n = 35) and 30% (n = 15), respectively. The allele frequencies for A and B allele were 95.25% and 4.75% in Jersey, 85% and 15% in HF and 54% and 46% in Kashmiri cattle, respectively. In view of the breed averages and preponderance of A allele in *Bos taurus*, it can be largely inferred that the polymorphism in 5′ flanking region of the α-La gene could be a potential molecular marker for production traits in dairy cattle. However, to authenticate it further evaluation through association studies are warranted.

1. Background

The last step in the biosynthesis of lactose in the mammary tissue is catalyzed by lactose synthase (LS). It is a heterodimeric protein complex comprising of α-La and β1, 4-galactosyltransferase (BGT) subunits (Voelker et al., 1997). Normally, BGT catalyzes the reaction between UDP-galactose and *N*-acetyl-D-glucosamine to form *N*-acetyl-lactosamine and UDP (Ramakrishnan and Qasba, 2001). The expression of α-La (synthesized specifically in mammary gland) increases dramatically at parturition and remains elevated throughout lactation (Brew et al., 1970). It is transported to the Golgi apparatus, where it binds to BGT and reduces the Km of BGT (1000 fold) for glucose to ~1 mM (within the physiological range of glucose concentrations) and thus the production of lactose ensues (Bell et al., 1976). In LS complex, the BGT and the α-La forms the catalytic and the modulator domain respectively (Pervaiz and Brew, 1985). Therefore, α-LA modifies the substrate (acceptor) specificity of BGT from NAG to glucose and results in the production of lactose and UDP (Brew et al., 1970; Ramakrishnan and Qasba, 2001). Lactose is the main osmole in milk which is incapable of passing through the lipid membrane of the secretory vesicles. In order to maintain osmotic equilibrium in secretory vesicles, it draws water into it by osmosis (Linzell and Peaker, 1971; Huang et al., 2012).

Abbreviations: α-La, α-Lactalbumin; BGT, β1, 4-galactosyltransferase; LS complex, Lactose synthase complex; SNP, Single nucleotide polymorphisms; HWE, Hardy-Weinberg Equilibrium

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Therefore, the concentration of α-LA in the mammary gland is conjectured to regulate milk volume by modulating the amount of lactose secreted into the milk (Linzell and Peaker, 1971; Kuhn et al., 1980; Boston et al., 2001). It has also been shown that α-LA is the limiting component of the LS complex (Grimble and Mansaray, 1987). Single nucleotide polymorphisms have gained importance in recent times because of their influence on the essential economic traits. It is well known that different breeds of cattle differ in the potential of production of quality and quantity of milk (Tetens et al., 2014).

Identification of SNPs, in genes responsible for milk quality and volume like α S1-casein, β-casein, α-La, and Pit-1 gene, etc. have been associated with quality and quantity of milk production (Lien et al., 1995; Renaville et al., 1997; Visker et al., 2011; Bonfatti et al., 2013; Zhou and Dong, 2013; Gustavsson et al., 2014). SNP at position + 15 of the α-LA gene was reported to be directly responsible for increased milk yield in two dairy herds and young sire population of Holstein breed (Bleck and Bremel, 1993; Mao, 1994; Martins et al., 2008; Schopen et al., 2011). Investigations also suggest a relationship between alteration at position − 1689 and the milk lactose concentration. Several SNPs in exon1 and exon2 have been reported from six indigenous breeds in Southern India (Visker et al., 2012). The present study was designed to explore the SNPs in the regulatory region spanning between − 363 to + 80 of the α-La genes in exotic high milk yielding breed like Jersey and HF, and indigenous low milk producing Kashmiri cattle. We calculate the frequencies of various observed alleles or haplotypes. The aim was to generate a baseline data for the variants to be used in future association studies which may help in establishing a breeding program based on molecular assisted selection for improvement of cattle livestock viz. a viz. milk production.

2. Material and method

2.1. Animals

For the present study, blood samples were collected from 50 unrelated HF breed of cattle maintained by Army farm, Bemina, Kashmir, 63 unrelated Jersey breeds of cattle maintained at Mountain Livestock Research Station, Manasbal, Sheri-Kashmir Institute of Agricultural Science-Kashmir (SKAUST-K), Kashmir and 49 indigenous unrelated Kashmiri cattle from various villages of district Anantnag, Kashmir. Genomic DNA was extracted from blood samples collected from the jugular vein in vacutainer pre-coated with heparin as an anticoagulant.

2.2. DNA samples

The DNA from 162 blood samples was extracted using the salting out method (Miller et al., 1988). The integrity of extracted DNA samples was checked by running it in 0.8% agarose gel. The DNA samples with intact bands were picked for further studies. The purity of the genomic extracted DNA was assessed by UV-spectrophotometer (Nanodrop 2000; Thermo scientific, USA) by recording optical density at 260 and 280 nm. Samples with 260/280 absorbance ratio between 1.7 and 1.9 were separated for further experimentation. Samples having 260/280 values below 1.7 were re-extracted till desired OD was attained. Similarly, the samples with 260/280 values above 1.9 were treated with RNAase and re-extracted.

2.3. DNA amplification by PCR

Based on published gene sequence for cattle (GeneBank accession no. U63109), a pair of PCR primers (F: 5′TGGACCCTTTGTGCATTTTCT3′ and R: 5′TGGGTGGCATGGAATAGGAT3′) was designed using primer 3 software to amplify sequence fragment of 5′ flanking region of the α-La gene. Primers were obtained from Sigma-Aldrich (P) Ltd., USA. The PCR fragment covered − 363 to + 80 bp region of α-La with a total of 443 bp fragment length.

The PCR was carried out in a total volume of 50 μl containing, 100 ng DNA template, 10 pM of each primer, 2 mM of dNTPs, 0.5 U of Taq DNA polymerase (Sigma-Aldrich), 10 × PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, $MgCl_2$) (Mullis, 1990). The PCR condition used for the amplification were as follows, initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s and lastly the final extension of 7 min at 72 °C. Amplified products were verified by electrophoresis of amplicons on 1% w/v agarose gel, in 1 × TAE buffer containing 0.5 μg/ml of ethidium bromide along with 1 kb DNA ladder (Fermentas) as a marker. The gel was visualized and documented using Gel documentation system (Gel doc EZ imager, Bio-Rad, USA).

2.4. Sequencing and sequence analysis

The amplified products were purified using column purification kit (Biotools B & M Labs, Spain) and all samples were sequenced by automated DNA Sequencer (ABI Genetic Analyzer, USA) following Sanger's Dideoxy Chain Termination procedure (Sanger et al., 1977). The sequences were analyzed using Finch T.V (PerkinElmer, Geospia, Inc.) Alignment reports were generated using Clustal W, sub-programme of Bio-Edit (Hall, 1999).

2.5. Statistical analysis

The frequencies of different genotypes and alleles were calculated using the standard procedure given by Falconer and Mackay (1996). The generated data was subjected to chi-square test to find out whether the studied populations were in Hardy-Weinberg Equilibrium (HWE) or not.

3. Result

3.1. PCR amplification and sequencing

The 443 bp promoter fragment corresponding to (− 363 to + 80) region of α-LA gene was amplified using DNA as a template obtained from in different breeds of cattle viz. Holstein (Fig. 1), Jersey (Fig. 2), Kashmiri samples (Fig. 3).

3.2. Sequence analysis

The sequencing results revealed three Genotypes AA, AB and BB coded by two different alleles ‘A’ and ‘B’ for the position − 250, − 89, and − 46. Three SNPs confirmed for the present genotypes at these positions are, G to A at position − 250, C to T at position − 89, and G to A at position − 46 for ‘A’ allele. For ‘B’ allele the changes were A to G at

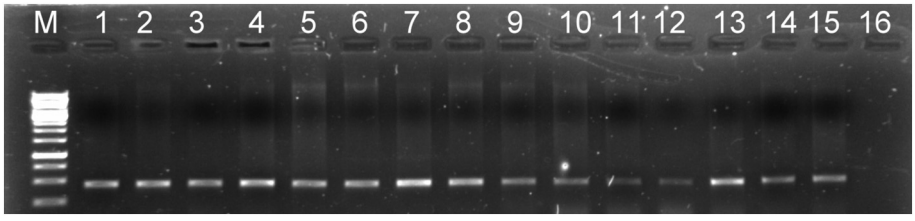

Fig. 1. Representative picture depicting electrophoretic pattern of amplified bovine α-La promoter using region specific primers encompassing 443 bp region (− 363 to + 80), separated on 1% agarose gel. Lane M: 1 kb DNA ladder marker. Lane1–15: Holstein breed samples, lane 16: negative control.