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Single nucleotide polymorphism in growth hormone gene exon-4 and exon-5 using PCR-SSCP in Black Bengal goats – A prolific meat breed of India

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Abstract

Single-strand conformation polymorphism (SSCP) showed 7 and 5 haplotypes in caprine GH gene exon-4 and exon-5 in Black Bengal, a prolific meat breed from India. All haplotypes revealed novel sequences. In exon-4 codons 6, 36 and 54 were polymorphic. At codon 6, AA arginine (R) changed to histidine (H) and proline (P), showing 6RR, 6HH and 6PP genotypes. At codons 36 three genotypes DD, VV and DV were observed due to SNP showing changed from aspartic acid (D) to valine (V). At codon 54, AA change from arginine to tryptophan (W) and 54RR and 54WW genotypes were observed. SNPs were also observed at codon 23 (serine to threonine) and at 37 (arginine to proline) in 8% of goats. In exon-5 nucleotide substitution (G/A) at codon 10 and (A/G) at 14 respectively changed AA from glycine (K) to glutamic acid (E). Silent mutations were also observed.

Keywords: Caprine; Growth hormone; Single-strand conformation polymorphism; Single nucleotide polymorphism; India

1. Introduction

Application of molecular genetics for genetic improvement relies on the ability to genotype individuals for specific genetic loci. Single nucleotide polymorphisms (SNP) are the class of direct markers that locate the loci that code for the functional mutation, have the edge over other markers, viz., linkage disequilibrium (LD) with functional mutations and population-wide linkage equilibrium (LE) markers (Andersson, 2001). Main application and potential for use of markers to enhance genetic improvement in livestock is through within-breed selection (Dekker, 2004). Selection of suitable candidate gene, depending on the trait under selection, is very important. First of all polymorphisms or markers need to be identified at the population level using random non-pedigreed samples. Analysis of marker-trait associations can bring a significant improvement for polygenic traits like milk yield, growth and meat production.

The Black Bengal goat is a major meat producing animal spread over vast geographic areas in the eastern region of India, Bangladesh and other Southeast Asian countries (Acharya, 1982; Mason, 1981). This breed presents a wide spectrum of variability in phenotypes, colour pattern, growth and body size, milk yield and reproduction rate across different regions (Bhattacharya, 2000). Despite higher fecundity, the Black Bengal goat generally has a relatively low milk production, which is often insufficient for feeding multiple kids (Chowdhury & Faruque, 2004). Improvement in milk production and growth rate is the

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major goal for which a suitable candidate marker needs to be identified that can be correlated within the breed.

Growth hormone (GH) is the major regulator of post natal growth and metabolism in mammals and thus affects growth rate, body composition, health, milk production and aging by modulating the expression of many genes (Chung, Kim, & Lee, 1998; Etherton et al., 1986; Ho & Hoffman, 1993; Lincoln, Sinowatz, el-Hifnawi, Hughes, & Waters, 1995; Sumantran, Thailand, & Schwartz, 1992). Growth hormone (GH) a single polypeptide hormone produced in the anterior pituitary gland is a promising candidate gene marker for improving milk and meat production in goats and other farm animals (Min, Li, Sun, Pan, & Chen, 2004). Growth hormone gene is encoded by 1800 base pairs (bp), consisting of five exons, separated by four intervening sequences (Gordon, Quick, Erwin, Donclson, & Maurer, 1983). The intragenic haplotypes of growth hormone gene in bovines have been described by Lagziel, Lipkin, and Soller (1996). Many polymorphisms have been identified in the regulatory (promoter), UTRs and exons of GH gene, but few of these have been precisely characterized for nucleotide changes and their positions in the DNA sequence.

Techniques have been described for the detection of point mutations in structural genes and their regulatory sequences. These are, denaturing gradient gel electrophoresis (DGGE) (Fischer & Lerman, 1980) and temperature gradient gel electrophoresis (TGGE) (Riesner et al., 1989). Single-strand conformation polymorphism (SSCP) is a powerful method, based on the differential migration of single stranded molecules through polyacrylamide gels based on the effect of sequence variation on intra stranded loop formation, for the identification of variations at a particular gene amplified product of DNA and has been used for the detection of genetic mutations in humans (Orita, Suzuki, Kanawaza, Sakiya, & Hyashi, 1989), rats (Pravenec et al., 1992) and in various bacteriological (Morohoshi, Hayashi, & Munakata, 1991) and viral strains (Fugita, Silver, & Pedea, 1992). The objective of this study was to identify the single nucleotide polymorphism (SNP) in growth hormone gene exon-4 and exon-5 in the Black Bengal goat breed, which would form the basis of a deeper study associating them with performance levels for improving milk and meat productivity of the breed.

2. Materials and methods

2.1. DNA extraction

Fifty "Black Bengal" animals (8 males and 42 females) from farmer's flocks were selected at random in the West Bengal (India) in 2004. Blood samples (10 ml) were obtained by jugular venipuncture, using vacuum tubes treated with 0.25% EDTA. DNA extraction was performed within 24 h according to Sambrook, Fristsch, and Maniatis (1989) with minor modifications and checked for quality and the quantity and was diluted to a final concentration of 50 ng/μl.

2.2. DNA amplification by PCR

Polymerase chain reaction (PCR) was carried out on about 50–100 ng genomic DNA in a 25 μl reaction volume. The primers described by Barracosa (1996) given in Table 1 of the exon-4 and exon-5 of growth hormone gene (Gene Bank D00476) of the goats were used (Kioka et al., 1989).

The amplified products of 225 bp and 365 bp respectively were expected to have a region from nucleotide 1396 to nucleotide 1620 of the exon-4 and from nucleotide 1795 to nucleotide 2159 corresponding to the exon-5 of caprine growth hormone gene. The reaction mixture consisted of 200 μM each of dATP, dCTP, dGTP and dTTP, 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.75 U Taq DNA polymerase and 4 ng/μl of each primer (Sigma Genosys), two drops of mineral oil, using PTC-200 PCR machine (M J Research Inc., MA, USA). Following a hot start (95 °C for 5 min), 30 cycles were carried out (95 °C for 30 s, 62 °C for 30 s, 72 °C for 30 s), ending with a 5 min final extension at 72 °C. Amplification was verified by electrophoresis on 2% (w/v) agarose gel in 1× TAE buffer using a 100 bp ladder (Invitrogen) as a molecular weight marker for conformation of the length of the PCR products. Gels were stained with ethidium bromide (1 μg/ml).

2.3. Single-strand conformation polymorphism analysis

PCR products were resolved by SSCP analysis. Several factors were tested for each fragment in order to optimize the amount of PCR products, denaturing solution, acrylamide concentration, percentage cross linking, glycerol, voltage, running time, temperature. Each PCR product was diluted in denaturing solution (95% formamide, 10 mM NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue, 20 mM EDTA) denatured at 95 °C for 5 min, chilled on ice and resolved on a polyacrylamide gel. The electrophoresis was carried out in a vertical unit (Bio-Rad Protean II xi), in 1X TBE buffer. Silver staining was as described by Sambrook et al. (1989). The gels were dried on cellophane paper using a gel dryer (Model 583, Bio-Rad).

2.4. PCR cleanup and DNA sequencing

The DNA samples showing different patterns on SSCP gels were selected for sequencing. The PCR products were purified by PCR purification kits (Biogene). The amplicons showing clear bands on agarose were further purified using Exo-SAP treatment in 96 well formats. Duplicate samples were chosen for each variant for both GH-4 and GH-5 segments of the caprine growth hormone gene. Primers used for sequencing were the same as those used for the