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Short communication

Polymorphism of exon 2 and 3 of growth hormone gene and presence of a rare genotype in native goat breed of Kerala, India

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ABSTRACT

Goats form an important livestock species in India, and is the only species showing an upward population trend in Kerala, the southernmost state of India. Reported polymorphism at 781A > G of Growth Hormone gene (*GH*) was screened. A total of 343 goats of two native breeds of Kerala namely, Malabari and Attappady Black, along with Malabari crossbreds formed the material for study. Restriction digestion with enzyme *HaeIII* resulted in three genotypes AA, AB and BB. Frequency for genotype AB was high for all populations, indicating its selective advantage, but an association between genotypes and growth traits could not be established. The BB genotype had a low frequency of 0.02 and appeared to be a rare genotype, which do not seem to be reported in other similar studies with *GH* gene. This genotype was seen mostly in Attappady Black goat population which comes under 'insecure' category of conservation and hence cannot afford to undergo strict selection measures, which might be the reason for the presence of this rare genotype.

1. Introduction

Goats, commonly described as 'Poorman's cow' form an important livestock species in India, mainly due to their high prolificacy, adaptation to local climatic conditions and acceptance of goat products by all communities. Goat is the only species showing an upward population trend in Kerala, the southernmost state of India. Native goat breeds of Kerala include the highly prolific Malabari goats and comparatively sturdy Attappady Black goats. Malabari goats are used for meat and milk purpose, and are seen in northern districts of Kerala. Attappady Black goats are noted for their hardy nature and disease resistant capacity, but have relatively low prolificacy and milk production capacity. Malabari crossbreds, a productive group of goats maintained in University Goat and Sheep Farm, Mannuthy, Kerala has the inheritance of native goat breed Malabari, and exotic goat breeds like Saanen, Alpine and Boer.

Growth Hormone gene (GH) is located at chromosome 19 q 22 in goats (Schibler et al., 1998; Pinton et al., 2000). Growth Hormone is an anabolic hormone synthesized and secreted by somatotroph cells of the anterior lobe of pituitary in a circadian and pulsatile manner (Ayuk and Sheppard, 2006) the pattern of which played an important role in growth and development, lactation, reproduction, as well as protein, lipid and carbohydrate metabolism (Akers, 2006; Supakorn, 2009). Kioka et al. (1989) reported that *GH* gene consists of five exons separated by four introns. Malveiro et al. (2001) and Marques et al. (2003) pointed out that exon 4 is more polymorphic than other exons of goat *GH* gene. High genetic variability in *GH* gene exon 4 and 5 were reported in Black Bengal, Sirohi and Jakhrana goats as several haplotypes were detected by PCR-SSCP study (Gupta et al., 2007, 2009).

Hua et al. (2008) assessed the association between different *GH* genotypes and growth traits. They reported that goats with AB genotypes at 781A > G locus (serine to glycine at residue 35) had a heavier body weight of 2 kg more than those with AA genotype at weaning. This transition from A to G destroyed restriction site recognized by endonuclease *HaeIII* resulting in PCR-RFLP polymorphism. Growth rate and prolificacy are two factors which determine the economy of goat rearing and hence require great attention. Reported polymorphism at 781A > G of *GH* was screened using restriction enzymes, to find out the frequency of alleles and genotypes in specific goat populations and to find out association with growth trait and prolificacy in goats. Absence of a particular genotype was reported by many workers and the present study aimed to evaluate whether this rare genotype was absent or present in native breeds of Kerala.

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2. Materials and methods

A total of 343 goats of Malabari, Attappady Black and Malabari crossbreds formed the material for study. Blood was collected from jugular vein of each animal into sterile 6 ml Ethylene diamine tetra-acetic acid (EDTA) coated vacutainers. The present study was approved by Faculty Research Committee as per order no: KVASU/DAR/R2/3892/11. Phenol chloroform method (Sambrook and Russell, 2001) was used to extract DNA from whole blood. Exon 2 and 3 of GH gene was amplified using published primers, which were custom synthesized after adopting from reference (Hua et al., 2008).

PCR was performed using the following ingredients, including 10 pM diluted primers, 200 μM of 10 mM dNTPs, 1 unit of Taq DNA Polymerase, 1.2 mM $MgCl_2$, 10× polymerase buffer and 100 ng of template DNA, made upto final concentration of 20 μl using ultra filtered Millipore water. PCR was done in Applied Bio-Systems thermal cycler, initial denaturation of 94 °C for 3 min, followed by 35 cycles of denaturation, annealing and elongation at 94 °C for 30 s, 62.2 °C for 30 s and 72 °C for 30 s respectively. Final elongation was done at 72 °C for 5 min.

Amplicons were outsourced for sequencing and the sequenced amplicons were evaluated using Basic Local Alignment Search Tool (BLAST) to ascertain the identity of gene segments and to locate the polymorphic sites if any, which formed the recognition site for restriction endonucleases. Thus *HaeIII* with recognition site GG▼CC was identified as restriction enzyme to type the polymorphism, G → A (Highlighted area is the polymorphic site and ▲ denotes cutting site for restriction enzyme). Restriction digestion of PCR products were performed at 37 °C, incubated for 15 min with one unit of enzyme. The samples after restriction digestion were checked in 2.0 percent agarose gels prepared in TBE and electrophoresed for one hour at 5 V/cm before being photographed in a Gel doc system (Bio-Rad, USA). Upon electrophoresis, the segments resolved in the gel and the genotypes were recorded.

Genotypic and allelic frequencies were calculated for Malabari, Attappady Black and Malabari crossbred goat populations under study. Genotypes were recorded after observing RFLP pattern, and frequencies were calculated by direct counting method (Falconer and Mackay, 1996). Allelic frequencies were calculated using Pop Gene V.32. Performance data were collected from 343 animals belonging to Malabari, Attappady Black and Malabari crossbred goats of University Goat and Sheep Farm, Mannuthy. Body weights and measurements at three months of age for growing animals and adult body weights of grown up animals were recorded for Malabari, Attappady Black and crossbred goats. Litter size of adult animals at different parities was obtained from farm records. For association study, subsets of this sample with complete records and genotype data were used. The associations for GH gene were worked out using General Linear Model (GLM) for non orthogonal data using SPSS V.21.

Model used for *GH* gene was

$$y_{ijkl} = \mu + b_i + r_j + g_k + e_{ijkl}$$

where y_{ijkl} = trait measured (length, girth, height, body weight or litter size) on ijklth animal

b_i = effect of ith breed, (i = 1 to 3), r_j = effect of jth birth weight, (*j* = 1 to 3, *i.e.* low, medium and high)

g_k = effect of kth *GH* genotype, (k = 1, 2, 3), e_{ijkl} = random error

3. Results

PCR specific for amplification of *GH* gene resulted in 422 bp fragment as seen in Fig. 1. Amplicons obtained from PCR were commercially sequenced and BLAST analysis with available sequences in NCBI database was performed to ascertain the identity of the fragment. Restriction digestion of 422 bp amplicon of *GH* gene, by endonuclease *HaeIII* resulted in three genotypes namely AA (366, 56 bp), AB (422, 366 and 56 bp) and BB (422 bp) (Fig. 1). Frequency for genotype AB was high for all the populations, indicating a selective advantage for AB genotypes in the population. Similar frequencies were reported by Hua et al. (2008) and Kumari et al. (2014). But according to them BB genotype was absent, whereas in the present study, BB genotype was present with very low genotype frequency (0.02).

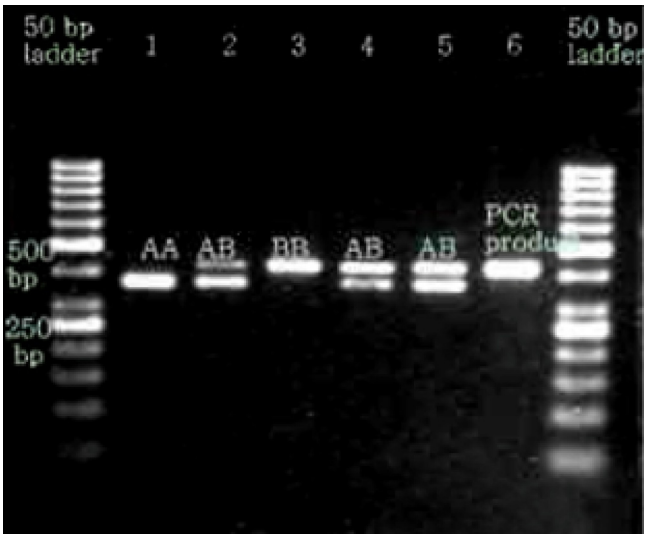

Fig. 1. PCR product and RFLP alleles of *GH* gene fragment resolved in 2% agarose gel. First and last lanes show 50 bp ladder, Lane 1–AA genotype, Lane 2, 4, 5–AB genotype, Lane 3–BB genotype and Lane 6–PCR product.

Table 1
Frequency of genotypes and alleles at growth hormone (*GH*) locus.

Population	GH genotype frequency			Allelic frequency	
	AA	AB	BB	A	B
Attappady Black (90)	0.08	0.88	0.04	0.52	0.48
Crossbred (101)	0.09	0.89	0.02	0.53	0.47
Malabari (152)	0.15	0.85	–	0.57	0.43
Overall (343)	0.11	0.87	0.02	0.55	0.45

Figures in parenthesis represent sample size.

AA and BB genotypes obtained from Malabari and Attappady Black goats respectively, were sequenced. Paired BLAST analysis of both AA and BB genotypes revealed two mismatches at 55th and 395th positions of 422 bp amplicon. The first mismatch 55G > A was found at the 90th position of exon 2 which is an established SNP (781A > G), producing aminoacid change from serine to glycine (AGC to GGC). BB sequence was submitted to GenBank, NCBI and Accession number obtained (KC7895171). This G > A transition, confirmed by chromatograms resulted in the disappearance of recognition site for *HaeIII* enzyme and hence the difference in banding pattern. Another novel SNP was noted at 395th position of amplicon (G > A), 42nd position of exon 3, which was a synonymous mutation as it did not produce any change in amino acid sequence of the protein. Frequencies of genotype and alleles for *GH* gene fragment presented in Table 1. BB genotype was absent in Malabari populations, and BB genotype frequency was extremely low in Attappady Black (0.04) and Crossbred (0.02) populations. Allelic frequencies for A and B alleles were almost equal (0.55 and 0.45 respectively).

Statistical analysis using GLM for non orthogonal data revealed no significant association for genotypes with body measure-