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## Single nucleotide polymorphism of growth hormone gene in Assam hill goats and their association with growth

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**Abstract**

The present investigation was carried out to study the occurrence of polymorphism in growth hormone (*GH*) gene in Assam Hill Goat and to associate polymorphic gene, if any, with body weights of the goats at 0 day, 3, 6, 9 and 12 months of age. A total of 256 blood samples were collected from male goats maintained at different field units and at the Goat Research Station, Assam Agricultural University, Burnihat under the "ICAR-All India Coordinated Research Project on Goat Improvement". The body weights of the same animals were recorded at 0 day, 3, 6, 9 and 12 months of age. PCR-RFLP analysis with *Hae* III restriction enzyme revealed two genotypes, viz. AA (25.39%) and AB (74.61%) with the gene frequency 0.63 (A) and 0.37 (B). The genotype frequency for AA and AB were found to be 0.40 and 0.60 respectively. Chi-square test revealed that the population was not in Hardy-Weinberg Equilibrium. The mean body weight at 0 day, 3, 6, 9 and 12 months of age for AA and AB genotypes were recorded to be  $1.18 \pm 0.03$  and  $1.16 \pm 0.02$ ,  $5.37 \pm 0.11$  and  $4.78 \pm 0.06$ ,  $7.50 \pm 0.12$  and  $7.14 \pm 0.08$ ,  $9.69 \pm 0.14$  and  $10.03 \pm 0.11$  and  $12.74 \pm 0.17$  and  $13.32 \pm 0.11$  kg respectively. However, statistical analysis did not show any significant difference ( $p > 0.05$ ) in body weights among the different genotypes.

**Keywords:** Assam hill goat, Growth hormone gene, Polymorphism, PCR-RFLP

**Introduction**

Eastern and North-Eastern part of India is rich in breeds of goat like Assam Hill goat, Sumi-Ne, Ganjam, Black Bengal etc which are known to be of good quality meat type. The goat meat does not have any social taboo and is accepted by all sections of people. Also, the meat contains low cholesterol and high level of iron and potassium as compared to other types of meat<sup>[1]</sup>. Assam hill goat is a native goat variety found in the state and is mainly reared for meat purpose and the chevon is of high quality. The reproductive traits of the goat breed include prolific nature of kidding, early sexual maturity and early kidding age. However, the Assam Hill goat is smaller in size with lower birth weight, weaning weight and matured body weight as compared to other breeds of goat found in India. Even though having good quality genetic resources may be, this is the reason behind the wide gap between the demand and the supply of meat in Assam. This gap could be bridged by enhancing the goat productivity by improving the growth performance as well as reproductive efficiency of the Assam Hill Goat. Molecular genetics has played a key role in discovering individual genes or candidate genes with substantial effects on the traits of economic importance. A total of 271 candidate genes have been detected in goats<sup>[2]</sup>. Growth hormone was one of the first genes to be used as a functional and positional candidate gene in genotype-to-phenotype association studies related to growth and carcass traits due to its role in postnatal growth, lactation, carbohydrate metabolism, and many other aspects of homeorhesis<sup>[3]</sup>. The caprine *GH* gene of 2544 bp consists of five exons and four introns<sup>[4]</sup> is mapped on chromosome 19<sup>[5]</sup>. The *GH* gene can accelerate metabolism and promote growth of many organs and tissues, especially the bone, muscle, and visceral organs. This gene produces the growth hormone, a peptide hormone, from the anterior pituitary, having implications in pre-natal growth<sup>[6]</sup> as well as for postnatal growth and metabolism in vertebrates<sup>[2, 7]</sup>. The function of Growth hormone gene has been intensively studied in cattle and pigs, while in goats the study is still rare. The objective of the present study was to determine polymorphism of GH gene in Assam Hill goats of Assam, India and to

associate 3<sup>rd</sup> polymorphic gene, if any with the body weights at 0 day and 3, 6, 9 and 12 months of age.

## Materials and Methods

### Location

Data pertaining to a total of 256 male goats were utilized in the present study. The goats were reared by the registered farmers of different field units as well as head quarter located at Goat Research Station, Assam Agricultural University under the ICAR sponsored project, "AICRP on Goat Improvement". The average temperature of the area ranged from 14.7 to 32.3°C and the relative humidity ranged between 51.2 and 93.7%. During the study period, the average annual rainfall was recorded to be 2818.0 millimetres. The location of the study lies between 90.48° and 92.22° (East) longitude and 20.09° and 26.95° (North) latitude.

### Body weight

With the help of weighing balance, the body weight of the male goats under the study was recorded at 0 day and 3, 6, 9 and 12 months of age. After the PCR-RFLP analysis of the amplicons, the goats were grouped according to their respective genotypes.

### Blood Collection and DNA Extraction

From the jugular vein of the goats under study, a volume of five ml of blood was collected aseptically in a vacutainer tube

containing 2.7% EDTA as an anticoagulant. The samples were brought from the field units to the laboratory in double walled ice-boxes containing ice packs and stored at -20 °C until the genomic DNA was extracted. Genomic DNA was isolated from the whole blood collected (5 ml) by following standard protocol [18] with slight modification. The purity of genomic DNA was assessed by Nanodrop Spectrophotometer (Picodrop, Model: Picopet01) and Optical Density (OD) values were measured at 260 and 280 nm which indicated the amount of DNA and the amount of protein in the given sample respectively. The concentration of genomic DNA was estimated spectrophotometrically by taking OD value at 260 nm. Horizontal submarine agarose gel electrophoresis was performed to check the quality of the isolated genomic DNA samples and was examined under gel documentation system (Gel Doc XR+, Bio-Rad). The quality of DNA was checked on 1.5% agarose gels stained with ethidium bromide.

### Amplification of GH Gene by PCR

The sequences of the forward and reverse primers for the amplification of the GH gene in the present study were:

Forward: 5'- TCCCTGCTCCTGGCTTTCAC -3' (Designed)

Reverse: 5'- GGAGAAGCAGAAGGCAACC-3' (Saleha *et al.* 2012) [19]

PCR was performed in a 25 µl reaction mixture containing 10.7 µl NFW; 0.4 µl Primer F; 0.4 µl Primer R; 12.5 µl master mix and 1µl of genomic DNA template. Thermal cycling conditions included:

Table 1.

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension
GH	94 °C for 4 minutes	94 °C for 30 seconds	54 °C for 30 seconds	72 °C for 45 seconds	72 °C for 5 minutes
		35 cycles			

The PCR products, thus obtained were separated and confirmed by horizontal submarine agarose gel electrophoresis (1.5%) using 100 bp DNA ladder 1X TAE buffer (80 V for 70 minutes). (Fig.1)

### Restriction Fragment Length Polymorphism (RFLP) analysis

The polymorphism was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The PCR products were digested with 0.4 µl *HaeIII* restriction endonuclease (Fermentas) at 37°C for 120 min followed by deactivation at 80°C for 20 min. This was subjected to 12% Polymerase Agarose Gel Electrophoresis (PAGE) carried out at 100 V for 2 hours, followed by staining with 0.5 µl of ethidium bromide. The digested fragments were visualized and documented using UV light and Gel Documentation System (Gel Doc XR+, Bio-Rad) and were analyzed by comparing with 50 bp DNA ladder. (Fig. 2)

### Sequence analysis

The PCR products were sent for sequencing to first base DNA sequencing division, Malaysia, which were sequenced by automated DNA sequencer following Sanger's dideoxy chain termination method [9]. The sequences were analyzed by using Clustal W method of DNASTAR Software (Lasergene, USA) to generate sequence alignment reports and residue substitution.

### Calculation of gene and genotype frequencies

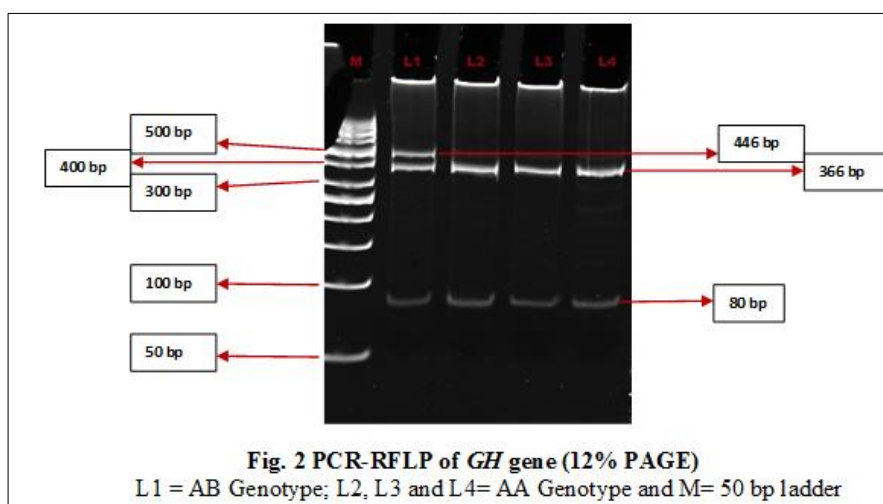
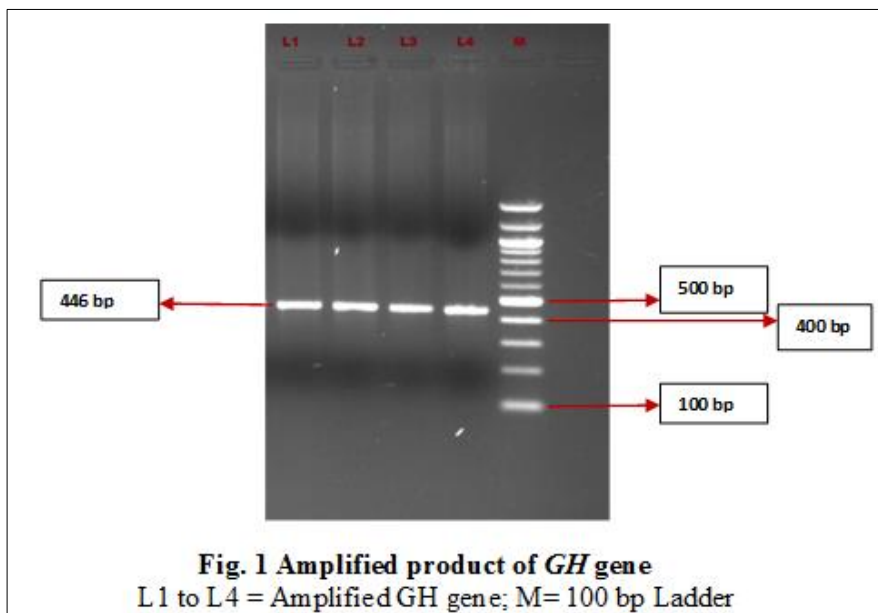
Gene and genotype frequencies were calculated using POPGENE 1.32 software [10].

### Statistical analysis

Statistical analysis to find the association between the polymorphic genes with the body weights at different ages were done by using SPSS software version 11.5. Chi-square statistic ( $\chi^2$ ) was used to check whether the populations were in Hardy-Weinberg equilibrium.

### Results and Discussion

The amplicons with the amplified fragments (Fig.1) of 446 bp when subjected to PCR-RFLP analysis produced two banding patterns, one with three and the other with two bands (Fig.2). Out of the 256 samples screened by PCR-RFLP analysis, 191 (74.61%) samples showed three bands (80 bp, 366 bp and 446 bp) referred as AB and 65 (25.39%) samples revealed two bands (80 bp and 366 bp) referred to be AA. The representative PCR amplicons of the respective banding patterns were sent for sequencing. These sequences were then aligned and compared using BLAST. The amplified region of GH among the screened samples revealed a distinct SNP (single nucleotide polymorphism) at 785<sup>th</sup> position (C→T). The frequencies of the alleles A & B were observed to be 0.63 & 0.37 and those of the genotypes AA & AB were found to be 0.40 & 0.60, respectively. The present findings were in accordance with that of other goats [11, 12, 13, 14, 15, 16]. However, in some other observations four different genotypes (AA, AB, CC and CD) could be found in respect of the GH gene [17, 18, 19].



The mean  $\pm$  SE body weights of goats belonging to AA genotype were recorded as  $1.18 \pm 0.03$ ,  $5.37 \pm 0.11$ ,  $7.50 \pm 0.12$ ,  $9.69 \pm 0.14$  and  $12.74 \pm 0.17$  Kg and that of AB genotype were  $1.16 \pm 0.02$ ,  $4.78 \pm 0.06$ ,  $7.14 \pm 0.08$ ,  $10.03 \pm 0.11$  and  $13.32 \pm 0.11$  Kg at the age of 0 day, 3, 6, 9 and 12 months respectively. Though the body weight at the same age groups of the two genotypes were found to have variations, the differences were found to be statistically non-significant ( $P > 0.05$ ). Present findings are in accordance with the findings of other goat breeds [12, 16, 20] who also could not establish any statistical significant relationship between the genotype of the GH gene detected and the body weights in Boer goats, Kacang goat and in Malabari and Attappady Black, along with Malabari crossbreds. However, statistically significant difference was observed between the genotypes and the body weights in different breeds of goats [17, 18, 21, 22, 23, 24, 25, 26, 27, 28]

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