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Single nucleotide polymorphism (SNP) detection in 5' flanking region of the growth hormone gene in Indian goat breeds

S KUMAR[1], S P DIXIT[2], M K VYAS[4], S C GUPTA[3] and JAGDEE KAUR[5]

National Bureau of Animal Genetic Resources, Karnal, Haryana 132 001 India

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Growth hormone (GH) secreted by the pituitary gland is the major regulator of postnatal growth and metabolism in mammals and thus affects growth rate, body composition, health, milk production and ageing by modulating the expression of many genes (Baldi 1999, Breier *et al.* 1999). There are many studies correlating milk (Lucy *et al.* 1993, Yao *et al.* 1996) and growth (Li *et al.* 2004) traits with polymorphisms at GH gene using single strand confirmation polymorphism (SSCP method. Single-strand conformation polymorphism (SSCP) is a powerful method for identifying sequence variation in amplified DNA because of one or more base changes. SSCP analysis of DNA has been used for detection of genetic mutations in humans (Oriia *et al.* 1989 a,b), rats (Pravenec *et al.* 1992), cattle (Kirkpatrick 1991), goat (Malverio *et al.* 2001, Marques *et al.* 2003) and chicken (Thakur *et al.* 2006). Hormones, growth factors and other regulatory proteins associated with so called "somatotropic axis" are candidate gene markers for quantitative traits in farm animals.

Genes encoding for growth hormone (GH) (Chitra and Aravindakshan 2004), GH receptor (GHR), transcription factor Pit-I (activating expression of GH and prolactin genes in the anterior pituitary), insulin-like growth factor-I (IGF-I), and perhaps genes coding for GH signal transduction pathways, could contribute to the progress in genetic selection of farm animals.

The aim of this study was to find out sequence variation in 5'upstream region growth hormone in 2 Indian goat breeds, using a non-radioactive PCR single-strand conformation polymorphism (PCR-SSCP) method, in order to find out new in/del or point mutations.

Sirohi (188) and Jamunapari (154), two breeds of Indian goat were used in this study. 10 ml of blood samples were collected from individual animals and genomic DNA was isolated from leukocytes using phenol-chloroform method (Sambrook *et al.* 1989) with minor modifications. Based on the published nucleotide sequence information of the goat GH gene (Accession no D00476, Kioka *et al*. 1989), the 5' up stream region was amplified by using primer pairs as follows: GH-PIF 5' cccagggattaaacctgagtc 3' and GH-PI R 5' etctgctgggccctttttat 3'.

With these primers, gGH amplification fragments were generated ranging in size from 13 to 364 bp which cover promoter binding site and up stream region. PCR reactions were performed using advanced primus 96 thermocycler according to the following conditions: 200 µM each of dATP, dTTP, dGTP and dCTP; 50 mM Kcl, 10 mM Tris-Hcl (PH 9.0), 0.1% Triton X-100, mM magnesium chloride; 0.75 unit of Taq DNA polymerase; 10 pM of each primers and 50–100 ng of genomic DNA for the final volume of 25µl. The amplification began with denaturation at 95°C for 30 s, annealing at 58°C for 30s, extension at 72°C for 30 s and final extension at 72°C for 5 min. The amplified products (5 µl) were detected on 2% agarose gel using 1 µl of loading dye as a stop dye, electrophoresed and visualized using UV light after ethidium bromide staining.

For single- strand conformation polymorphism (SSCP) analysis, 5µl of each amplification product was added to 10–15 µl of stop solution (95% formamide, 10 mM NaOH, 0.05% xylene cylanol and 0.05% bromophenol blue, 20mM EDTA) and denatured at 95°C for 5 min, snap cooled on crushed ice. 15–20 µl of each sample solution was loaded on 8–12% non-denaturizing polyacrylamide/TBE gel. Gels were run at 25 W for 4–8 h depending upon the product length at 10–15°C in a Universal mutation detection system (BIO-RAD) coupled with refrigerated system. After the run, the gel was removed from the apparatus and the DNA bands were visualized through silver staining method.

The studies of genetic marker applied to animal breeding and production is focused mainly on analyses of mutations located within candidate genes and searching association with quantitative traits. We used the PCR-SSCP method to identify

Present address: [1]Research Associate, [2]Senior Scientist (e mail: dixitsp@gmail.com), [4]SRF, NDRI, Karnal.

[5]Associate Professor, Department of Biotechnology, Punjab University, Chandigarh.

[3]ADG (Animal Science), Indian Council of Agricultural Research, New Delhi 110 114.

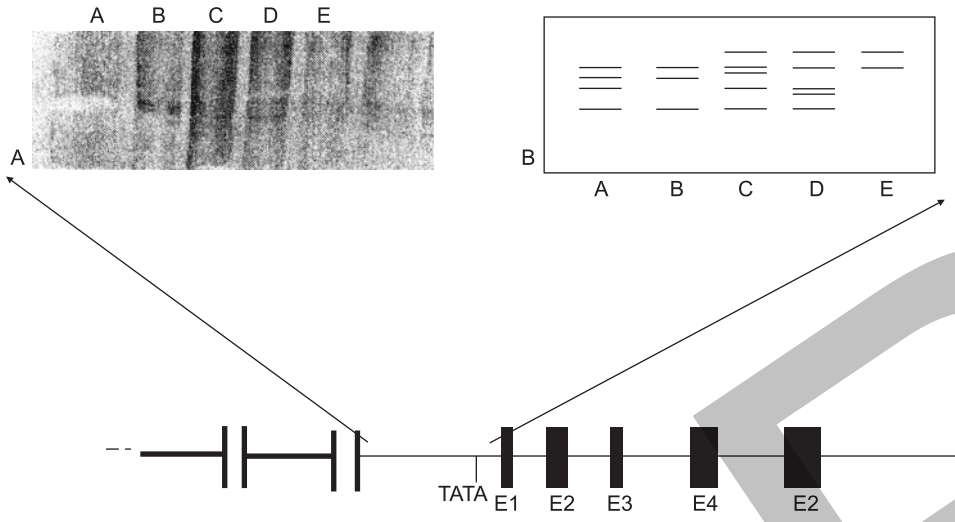

Fig. 1. SSCP patterns of 5' region of goat growth hormone gene (gGH) separated by non-denaturing PAGE. **A**- Polyacrylamide gel electrophoresis of 352 bp gGH gene fragments amplified from DNA of 342 animals. **B**-Schematic representation of individual SSCP pattern. Schematic representation of goat GH gene. Exons (E) are represented by black boxes.

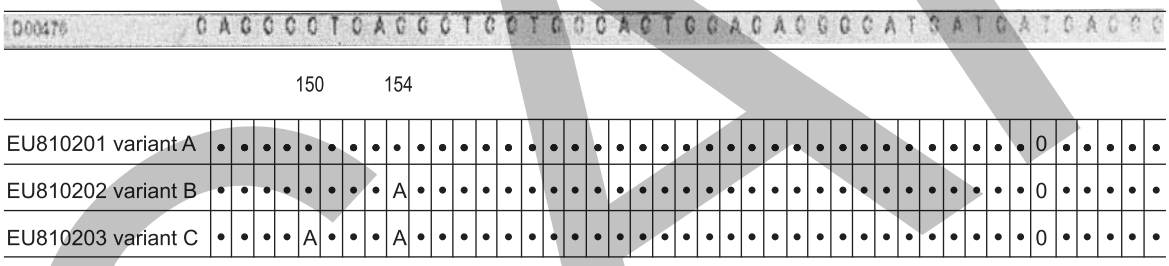

Fig 2. Comparative alignment of the PCR-SSCP haplotypes sequence of 354 nucleotides (only 5' region of growth hormone gene in the Indian goat) with Gene reference sequence D00476 based on Megalign module of DNA star software version 4.0. Nucleotide positions 150, 154 and 183 show nucleotide substitution.

Table 1. (A) SSCP analysis parameters and frequencies of 5 different variants in 5' up stream region of gGH gene

Fragment	p.100T	Run Temp (°C)	Number of patterns (breed)	Frequency of patterns (%)				
13–364	12	10	5	A	B	C	D	E
			Sirohi	1.06	39.35	38.29	12.23	05.31
			Jamunapari	19.76	43.46	27.26	33.74	-

sequence variants in 5' upstream region of growth hormone in Indian goats breed. SSCP variants and their frequencies in growth hormone gene in Sirohi and Jamunapari breeds of goat are presented in Table 1 and Fig. 1. The SSCP analysis of the gGH gene revealed high level of polymorphism at 5' region in these breeds of goat. Five reproducible SSCP pattern in Sirohi and 4 in Jamunapari breed of goat were detected. The sequence analysis revealed 3 possible substitution mutations, 2 transition substitutions at position 183 T>C, 154 G>A and 1 transversion substitution 150 C>A. All the sequences (354 nucleotides sequence) were novel and distinct from with Gene reference sequence D00476 based on Megalign module of DNA star software version 4.0 (Fig. 2). The unique 3 sequences were deposited in the NCBI Genebank (Accession No. EU810203). The single nucleotide polymorphism was also reported in the promoter region of growth hormone gene in Boer goats (Li *et al.* 2004).

This study showed the polymorphic nature of the 5' region of goat growth hormone gene. The difference in genotype frequency of SSCP pattern and absence of some SSCP patterns between goat breeds may be due to stochastic factors such as genetic drift and founder group effect. The data generated by current studies may be useful for establishing possible associations between productive parameters and genetic variants and help in the process of decision making at the farmers level for improvement and sustainable

management of these goat breeds.

SUMMARY

Polymorphism in the 5' region of growth hormone (GH) gene in 2 Indian goat breeds, viz. Sirohi and Jamunapari, was investigated using an optimized non-radioactive polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) method. Based on the published nucleotide sequence information on goat growth hormone (Gene Bank D00476), oligonucleotide primer was designed to amplify a 352 bp covering 13 to 364 nucleotide (nt) sequence region. The PCR product was denatured and subjected to polyacrylamide gel electrophoresis to detect SSCP and 5 reproducible patterns were found. Out of these patterns, 3 possible substitution mutations were identified at position 183 T>C, 154 G>A and 150 C>A. The results confirmed that there were polymorphisms in the 5' up stream region of the goat growth hormone gene. Further studies need to be carried out to verify their effects on the expression of GH gene and their association with production traits.

REFERENCES

Baldi A. 1999. Manipulation of milk production and quality by use of somatotropin in dairy ruminants other than cow. *Domestic Animal Endocrinology* **17**: 131–37.

Breier B H. 1999. Regulation of protein and energy metabolism by the somatotropic axis. *Domestic Animal Endocrinology* **17**: 208–09.

Chitra R and Aravindakshan T V. 2004. Polymorphism at growth hormone gene in Malabari goats investigated by PCR-RFLP. *Indian Journal of Animal Sciences* **74**: 1215–18.

Kioka N, Manabe E, Abe M, Hashi H, Yato M, Okuno M, Yamano Y, Sakai H, Komano T, Utsumi K and Iritani A. 1989. Cloning and sequencing of goat growth hormone gene. *Agricultural and Biological Chemistry* **53**: 1583–87.

Kirkpatrick B W, Cowan C M and Dentine M R. 1991. Differential amplification of alleles: potential for misclassification with PCR genotyping. *Animal Biotechnology* **2**: 1–4.

Li M, Ei Yu, Min Lingjiang Pan Qingjie, Sun GuoQiang. 2004. Study of Polymorphism of Goat GH gene and its association with body weight. *Animal Biotechnology Bulletin* **9**: 176–80.

Lucy M C, Hauser S D, Eppard S D, Krivi P J, Clark G G, Baumann D E and Collier R J. 1993. Variation of somatotropin in cattle: gene frequency in major dairy breeds and associated milk production. *Domestic Animal Endocrinology* **10**: 325–33.

Malveiro E, Pereira M, Marques P X, Santos I C, Belo C, Renaville R and Cravador A. 2001. Polymorphisms at the five exons of the growth hormone gne in the algarvia goat: possible association with milk traits. *Small Ruminant Research* **41**: 163–70.

Marques P X, Pereira M, Marques M R, Santos I C, Belo C C, Reuaville R and Cravodor A. 2003. Association of milk trait with SSCP polymorphism at growth hormone gene in Serana goat. *Small Ruminant Research* **50**: 177–85.

Orita M, Suzuki Y, Sekiya T and Hayashi K and Sekiya T. 1989a. Detection of polymorphism of human DNA by gel Electrophoresis as single strand conformation polymorphism. *Proceeding of National Academy of Science of the USA* **86**: 2766–70.

Orita M, Suzuki Y, Sekiya T and Hayashi K. 1989 b. Rapid and sensitive detection of point mutation and DNA polymorphisms using polymerase chain reaction. *Genomics* **5**: 874–79.

Pravenec M, Simonet L, Kren V, St. Lezin E, Levan G, Szpirer C and Kurtz T. 1992. Assignment of rat linkage group V to chromosome 19 by single strand conformation polymorphism analysis of somatic cell hybrids. *Genomics* **12**: 350–56.

Sambrook J, Fritsch E F and Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd edn. Cold Spring Harbour, Cold Spring Laboratory Press, NY.

Thakur M S, Parmar S N S, ToJenkhomba T C, Srivastava P N, Joshi C G, Rank D N, Janki V S J and Pillai P V A. 2006. Growth hormone gene polymorphism in Kadaknath breed of poultry. *Indian Journal of Biotechnology* **5**: 189–94.

Yao J, Aggrey S E, Zadworny D, Hayes J F and Kuhnlein U. 1996. Sequence variation in the bovine growth hormone gene characterized by single strand conformation polymorphism (SSCP) analysis and their association with milk production traits in Holsteins. *Genetics* **144**: 1809–16.