Detection of Polymorphism of Growth Hormone Gene for the Analysis of Relationship between Allele Type and Growth Traits in Karan Fries Cattle

Aruna Pal, A. K. Chakravarty, T. K. Bhattacharya[1,] *, B. K. Joshi and Arjava Sharma[1]
Dairy Cattle Breeding Division, National Dairy Research Institute, Karnal, Haryana-132 001, India
[1] Animal Genetics Division, Indian Veterinary Research Institute, Izatnagar, U.P., India.

ABSTRACT : The present study was conducted to detect polymorphism at growth hormone gene in Karan Fries bulls. A 428 bp fragment of growth hormone gene spanning over 4th exon, 4th intron and 5th exon was amplified and digested with *Alu*I restriction enzyme to identify polymorphism at this locus. Karan Fries bulls were found to be polymorphic at this locus. Two genotypes LL and LV were identified in Karan Fries with higher allelic frequency for L allele. In Karan Fries males, the average birth weight, 3 months body weight and daily body weight gains of LL homozygotes were significantly higher than that of LV heterozygotes. Genetic distances of KF bulls with respect to genotype along with 3 months body weight and average daily body weight gain forms a single cluster of bulls with LL genotype, while individuals with LV genotype forms three distinct clusters indicating more influence of L allele on growth traits. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 10 : 1334-1337)***

Key Words : Association, Birth Weight, Cattle, Body Weight, Growth Hormone Gene, Polymorphism

INTRODUCTION

Association of polymorphic candidate gene with economic traits will help the breeders to search some genetic marker for economic traits. This may be used as an aid to the selection of bulls at an early stage and can save huge economic loss for rearing the bulls till maturity. Growth hormone helps in body growth and metabolism through protein synthesis, protein deposition in tissues and organs (Gluckman et al., 1987), thus Growth Hormone leads to increased nitrogen retention (Hart and Johnson, 1986), gluconeogenesis and cell division (Neathery et al., 1991). Growth hormone increases intestinal calcium absorption, thereby enhancing overall bone growth and stimulating chondrocyte proliferation, (Boyd and Baumann, 1989). Growth hormone is a polypeptide hormone with 191 amino acid sequences. Growth hormone gene has been assigned to 19q26qter position of bovine chromosome (Hediger et al., 1990) with five exons and four introns. Fifth exon of the growth hormone gene at 127 amino acid position was found to be polymorphic with two allele L and V corresponding to Leucine and Valine variant of growth hormone polypeptide, respectively. This variation was due to C to G substitution at growth hormone gene, which was detected by *Alu*I RFLP. Earlier, some workers reported that genotype of growth hormone gene was associated with birth weight in dairy cattle (Biswas et al., 2003) and mature live weight in beef cattle (Zwierzchowski et al., 2001). However, studies on the association of different growth characteristics with the growth hormone gene polymorphism are scanty whereas such type of marker trait association studies have also been documented by a number of workers in various ETLs (Badola et al., 2003). Hence, the present investigation was carried out to find out the polymorphism of growth hormone gene and its association with growth characters like body weight at birth, three months and average daily body weight gain of both cattle and buffalo.

* Corresponding Author: T. K. Bhattacharya. Tel: +91-581-2303382, Fax: +91-581-2303284, E-mail: tarunivri@yahoo.co.in
Received October 15, 2003; Accepted May 25, 2004

MATERIALS AND METHODS

Animals

The present study was carried out in twenty-six Karan Fries (Holstein Friesian×Tharparkar) bulls maintained at Artificial Breeding Complex of the National Dairy Research Institute, Karnal, Haryana, India.

Sample and data

About 10ml venous blood was collected under sterile conditions from the jugular vein of the animals into a sterile 50 ml polypropylene vial containing 0.5 M EDTA as anticoagulant. Data on birth weight, three months body weight and daily body weight gain were collected from Record Section of Dairy Cattle Breeding Division, National Dairy Research Institute, Karnal, Haryana, India.

DNA preparations

Genomic DNA was isolated from blood samples following phenol- chloroform extraction method described by Sambrook et al. (1989). After isolation, DNA pellet present in eppendorf tube was dissolved in TE buffer and was kept in water bath at 60°C for 2 h to dissolve pellet properly in buffer. DNA quality was checked by spectrophotometry. DNA with O.D. ratio between 1.7 and

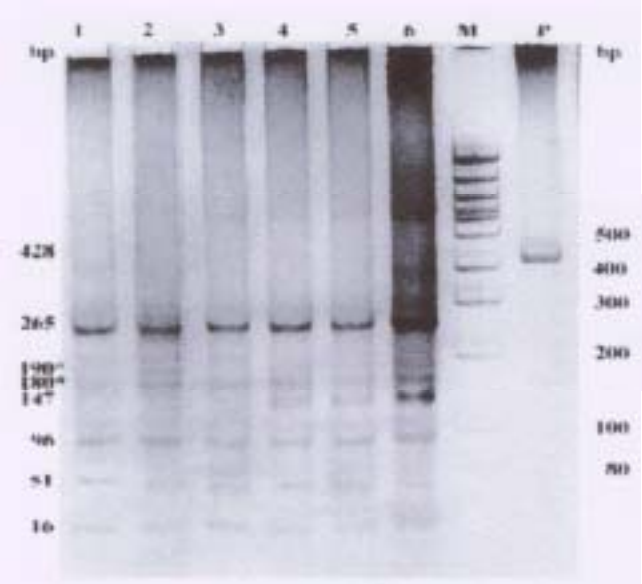

Figure 1. *Alu*I RFLPs of Growth hormone gene in Karan Fries bull. Lane 1, 2 and 3 are LL genotype; lane 4, 5 and 6 are LV genotype; lane M is 100 bp DNA ladder; lane P is PCR product. * Indicates the presence of nonspecific bands.

1.9 were considered to be of good quality and used for further study. The samples beyond this range were re-extracted with Phenol-chloroform extraction method.

DNA amplification

A 428 bp fragment of growth hormone gene spanning over 4th exon, 4th intron and 5th exon was amplified with the forward (5′-CCGTGTCTATGAGAAGC-3′) and reverse (5′-GTTCTTGAGCAGCGCGT-3′) primers. PCR was carried out in a final volume of 25 µl reaction mixture containing 80-100 ng DNA, 2.5 µl 10X PCR assay buffer, 200 mM of each dNTP, 3 U Taq DNA polymerase, 20 pmM of each primer and 2 mM $MgCl_2$. To check contamination, a negative control, labelled 'C' with master mix devoid of template DNA was made. Then, PCR tubes were kept in a pre-programmed thermocycler (PTC-200, MJ Research, USA) for amplification.

Two stage PCR programmes were followed to obtain the optimum PCR yield. In the first stage, the cycling conditions were at 94°C for 5 min followed by 10 cycles of denaturation at 94°C for 1 min, annealing at 46°C for 45 sec, extension at 72°C for 50 sec. In last stage, 25 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 45 sec were carried out, followed by final extension at 72°C for 50 secs.

RFLP and Polyacrylamide gel electrophoresis

The 428 bp amplicon was treated with *Alu*I enzyme to identify polymorphism at Growth hormone gene. A volume of 20 µl of PCR product was digested with 10 U *Alu*I enzyme and 10X assay buffer at 37°C for overnight. The reaction was stopped by adding 0.5 M EDTA.

The digested product was separated on an 8% non-denaturing polyacrylamide gel at 100 V for 5 h. The gel was stained with silver nitrate, as described by Bassam et al. (1991), with slight modifications where 10% glacial acetic acid was used for fixing the DNA bands for 30-45 min, then stained with 0.1% silver nitrate for 30 min. Three percent sodium carbonate containing 300 µl of formalin was used as a developer. After staining, the fragments were visualized in gel doc system.

Estimation of the size of the fragments

The length of each fragment generated by *Alu*I restriction enzyme digestion were compared with the markers (100 bp DNA ladder loaded in a separate lane in the same gel) and the fragment size was estimated using the formula, D=a-b log M, where D stands for distance migrated by a particular fragment, M stands for the mass of that fragment. The two constant 'a' and 'b' specific for each gel were calculated by equating a regression equation each for known fragments length or mass with known distance migrated.

Statistical analysis

Gene and genotype frequency was estimated following the method described by Falconer and Mackay (1996). Chi-square test was used for finding the effect of genotype on growth characteristics. Using Euclidean distance with single linkage (Jobson, 1992), the animals were classified corresponding to the genotypes and the growth characteristics of male animals to find out whether a particular genotype of GH gene was similar or dissimilar with respect to growth traits.

RESULTS

Genotyping

The PCR amplification generated a 428 bp segment from growth hormone gene in cattle, thus it indicates strong conservation of DNA sequence being existed in the species whichever is the breed is. The primers were basically of cattle origin but because of very high degree of nucleotide sequence conservation in cattle, these primers are likely to give similar size of amplification product (Figure 1).

In Karan fries cattle, two different restriction patterns were obtained corresponding to two different genotypes LL and LV. Five fragments of 265 bp, 147 bp, 96 bp, 51 bp, 16 bp were found in individuals with LV genotypes whereas four restriction fragments with 265 bp, 96 bp, 51 bp, 16 bp were observed in LL genotype.

Gene and genotype frequency

In Karan Fries bulls, the genotypic frequency of LL homozygotes was found to be 0.115, whereas genotypic

Table 1. Genotype-wise performance of Karan Fries bulls

Species	Genotype	Birth weight (kg)	3 M Body weight (kg)	Average daily body weight gain (gm)
Cattle (KF)	LL	28.83±1.13[a]	59.38±2.67[a]	589.99±21.77[a]
	LV	24.00±2.52[b]	56.67±5.46[b]	562.96±52.24[b]

Different superscripts indicate significant differences at p≤0.01.

frequency of LV heterozygotes was 0.884. Allelic frequency for L allele was 0.94, whereas that of V allele was 0.06. Thus, the frequency of L allele was found to be more than that of V allele.

Effect of genotype

In Karan Fries bulls, genotype of growth hormone gene had a significant effect on birth weight, three months body weight and average daily body weight gain. In Karan Fries bulls, LL homozygotes had significantly higher average birth weight (p≤0.01) than that of KF bulls having LV genotype. In crossbred animals, the LL homozygotes had significantly higher (p≤0.01) three months body weight and average daily body weight gain than that of LV heterozygotes (Table 1).

Genetic distance study revealed that among all the growth traits, body weight at three months and average daily body weight gain of the Karan Fries males were responded distinctly corresponding to the genotypes of growth hormone gene. The cluster tree formed using the genotype of growth hormone gene along with the identified growth characteristics are presented in Figure 2. Distance studies have shown that in Karan Fries bulls, twenty-three LL homozygotes were similar with respect to body weights at three months and average daily body weight gain. On the otherhand, LV heterozygotes were clustered in three distinct clusters, indicating that these bulls were dissimilar with respect to genotype of growth hormone gene, body weight at three months and average daily body weight gain.

DISCUSSION

For growth hormone loci, Karan Fries cattle were found to be polymorphic. The two types of alleles differ only in terms of restriction site of *Alu*I endonuclease enzyme. The L allele indicated the presence of restriction site while its absence was assigned as allele V. In L allele the restriction site contained the nucleotide C while a transition with G at the same site indicated the absence of *Alu*I restriction site. The total length of amino acid in growth hormone is 191. The presence of nucleotide C at triplet codon encodes the amino acid leucine while the nucleotide; G encodes the amino acid valine. This leucine/valine substitution was found in 127th position of the polypeptide.

In Karan Fries cattle, the frequency of L allele was found to be higher than that of V allele, and correspondingly frequency of LL genotype was more than that of LV genotype. Thus the present result was in agreement to the finding of Lucy et al. (1993), and Lechniak et al. (1999), who have also reported similar higher gene frequency of L allele in the dairy bulls. Karan fries is the crossbred cattle evolved through crossing between Holstein-Friesian (Exotic) Tharparkar (Indigenous). Earlier studies (Lucy et al., 1993; Biswas et al., 2003) reported that the frequency of 'L' allele in both Holstein Friesian and Holstein Friesian crosses were very high. Biswas et al. (2003) reported that the frequency of 'L' allele in Holstein Friesian cattle was as high as 0.85. They also delineated the monomorphism of Growth Hormone gene with predominance of LL genotype in Indigenous cattle. So, it was possible to have only LL and LV genotypes in crossbred animals. Although there were some reports in allelic variability in different cattle breeds, but there is a quite less chances to have nucleotide variability in exon region of growth hormone gene. Growth hormone as a vital endocrine secretion is mostly conserved in nature as far as polypeptide sequence is concerned. It indirectly reflects the conservation of nucleotide sequence of the gene more specifically coding region. However, nucleotide sequence of growth hormone gene in cattle was reported by Gordon et al. (1983). Nucleotide alignment of cattle and human growth hormone gene showed enormous conservation between them (Gordon et al., 1983).

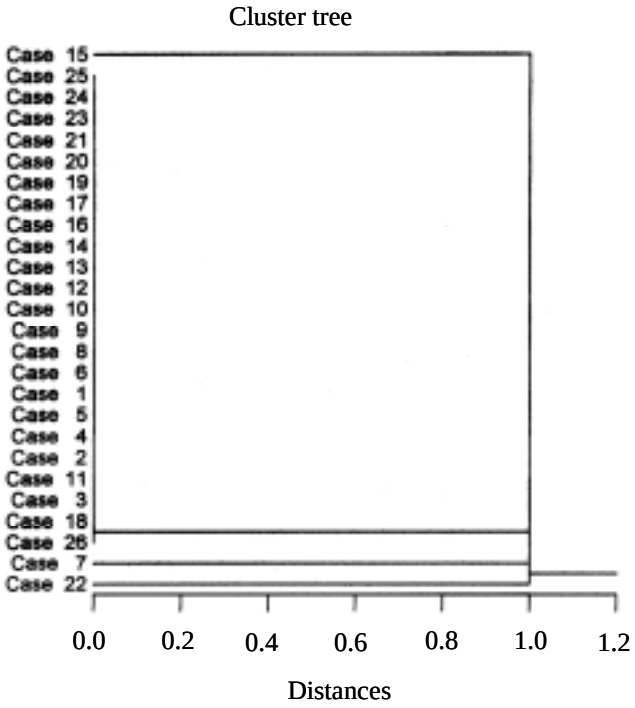

Figure 2. Cluster tree of twenty-six KF bulls based on Euclidean distance, Cases indicate the respective bulls.

The genotype of growth hormone gene had a significant effect on birth weight, three months body weight and average daily body weight gain in Karan Fries bulls. The average birth weight of LL homozygote was significantly higher ($p<0.01$) than LV heterozygotes by about 20.13 percent. The finding is in contrast to Biswas et al. (2003) where they found LV genotype favoured higher birth weight in Holstein-Friesian cattle.

The average three months body weight and daily body weight gain of LL homozygotes in Karan Fries bulls were significantly higher than that of LV heterozygotes by about 4.78 and 4.80 percent, respectively. This finding is in contrast to the studies conducted by Zwierschowski et al. (2000) who have reported VV homozygotes having higher live weight, however they had studied beef cattle.

The cluster tree analysis also conformed our results of association study estimated by chi-square test. The distance values derived from cluster trees based on genotypes and growth traits truly depict the significant effect of genotype on growth parameters. But, the analysis on large number of animals needs to be done to confirm the positive relationship between polymorphism of growth hormone gene with different growth characters.

REFERENCES

Badola, S., T. K. Bhattacharya, T. K. Biswas, P. Kumar and A. Sharma. 2003. Association of beta-lactoglobulin polymorphism with milk production traits in cattle. Asian-Aust. J. Anim. Sci. 16(11):1560-1564.

Bassam, B. J., G. Gaetano-Anolles and P. M. Gresshoff. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Analytical Biochem. 196:80-83.

Biswas, T. K., T. K. Bhattacharya, A. D. Narayan, S. Badola, P. Kumar and A. Sharma. 2003. Growth hormone gene polymorphism and its effect on birth weight in cattle and buffalo. Asian-Aust. J. Anim. Sci. 16(4):494-497.

Boyd, R. D. and D. E. Baumann. 1989. Mechanism of action of somatotropin in growth. In: Current Concepts of Animal Growth Regulation (Ed. D. R. Campion, G. J. Haeesman, R. J. Martin). Plenum Press, New York, pp. 257-293.

Falconer, D. S. and T. F. C. MacKay. 1996. Introduction to quantitative genetics, 4th Edn. Longman Group Ltd., Essex. England.

Gluckman, P. D., B. H. Breier and S. R. Davis. 1987. Physiology of the somatotropic axis with particular reference to the ruminant. J. Dairy Sci. 70:442-466.

Gordon, D. F., D. Quick, C. R. Erwin, J. Donelson and R. A. Maurer. 1983. Nucleotide sequence of the bovine growth hormone chromosomal gene. Mol. Cell. Endocrinol. 33:81-95.

Hart, I. C. and I. D. Johnson. 1986. Manipulation of milk yield with growth hormone. In: Recent Advances in Animal Nutrition (Ed. W. Haresign and D. J. A. Cole). Butterworths, London, UK.

Hediger, R., S. E. Johnson, W. Barendse, R. E. Drinkwater, S. S. Moore and J. Hetzel. 1990. Assignment of the GH gene locus to 19q26qter in cattle and to 11q25qter in sheep by *in situ* hybridization. Genomics 8:171-174.

Lechniak, D., G. Machnik, M. Szydlowski and M. Switonski. 1999. Growth hormone gene polymorphism and reproductive performance of AI bulls. Theriogenology 52:1145-1152.

Jobson, J. D. 1992. Applied multivariate data analysis, Vol.2. Published by Springer-Verlag, New York Inc.

Lucy, M. C., S. D. Hauser, P. J. Eppard, G. G. Krivi, J. H. Clark, D. E. Bauman and R. J. Collier. 1993. Variants of somatotropin in cattle : Gene frequencies in major dairy breeds and associated milk production. Domestic Anim. Endocrinol. 10:325-333.

Neathery. M. W., C. T. Crowe, G. F. Hartnell, J. J. Veensuizen, J. O. Reagan and D. M. Blackmon. 1991. Effects of sometribove on performance, carcass composition and chemical blood characteristics of dairy calves. J. Dairy Sci. 74:3933-3939.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular cloning - A Laboratory Manual. 2nd Edn., Vol.3, Cold Spring Harbour Laboratory Press, pp. 6:3-4.

Zwierzchowski, L., J. Oprzadek, E. Dymnicki and P. Dzierzbicki. 2001. An association of growth hormone, kappa-casein, beta-lactoglobulin, leptin and pit-1 loci polymorphism with growth rate and carcass traits in beef cattle. Anim. Sci. Papers and Reports 19:65-77.