DHR International Journal Of Biomedical and Life Sciences (DHR-IJBLS)
ISSN 2278-8301, Vol. 4(2), 2013
Available online *http://www.doublehelixresearch.com/DHRIJBLS*

Original Article

Polymorphism Studies of Growth Hormone Receptor (GHR) Gene in Indigenous Grey Cattle Breeds of India

Deepika[1, 2]* and Raj Kumar Salar[3]

[1] National Bureau of Animal Genetic Resources, Karnal-132001, Haryana, India
[2] University College, Kurukshetra University, Kurukshetra-132119, India
[3] Department of Biotechnology, Chaudhary Devi Lal University, Sirsa-125055, India
*Corresponding author: Deepika

Abstract

The present study was undertaken to detect polymorphism in Exon 10 and 5' non-coding regions of GHR gene that have been reported to be associated with meat and milk related traits in 10 indigenous grey cattle breeds from different agro-climatic regions of India. Fragments of 462 bp from exon 10 and 302 bp from 5' non-coding regions of GHR gene were amplified using specific primer pairs and digested by using *Alu*I and *Nsi*I restriction enzymes. Of the 453 animals screened, 79.2% were homozygous for AA genotype and 20.8% were heterozygous for AG genotype. No GG homozygous animal was observed. For the second set of primer out of 451 animals screened 94.2% animals were homozygous for AA genotype, 5.6% of animal were homozygous for GG genotype and 0.2% animals were heterozygous (AG genotype). The frequency of G allele was significantly lower than the A allele in all the cattle breeds.

Key words: Growth hormone receptor; Polymorphism; Milk; Traits.

Introduction

The productive traits in livestock like growth, carcass, milk yield and composition, Growth hormone receptor (GHR) is important candidate gene for the identification of genetic markers. In cattle, the GHR gene is located on chromosome 20 [6]. In the structural or regulatory sequences of growth hormone and its receptor genes, any allelic variation might directly or indirectly affect milk traits. This possibility prompted us to investigate the restriction fragment length polymorphism at the locus of bovine growth hormone receptor, which may serve as genetic marker. Several polymorphic sequences and their association with milk and meat

production traits have been identified in the bovine *GHR* gene [1, 2, 3, 4, 5, 8, 10, 11, 12, 13, 14 and 15]. The same polymorphisms were analyzed in the ten cattle breeds by PCR-RFLP. Thus Growth hormone receptor genes has been shown to be directly associated with milk and growth of the individuals and thus are important candidates for analysis. The objective of this study was to identify and characterize polymorphisms in the GHR genes in native indigenous grey cattle breeds and compare its pattern with taurine cattle. It is hoped that the present study will contribute to the scientific basis for the selection parameters of native breeds.

Materials and Methods

Fresh Blood samples (7-8 ml) were collected from 40-50 genetically unrelated animals of ten cattle breeds from their respective native breeding tracts. Blood samples were collected by jugular vein puncture, using Vacutainer® blood collection tubes treated with 0.25% ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The blood samples were transported from the field to laboratory and genomic DNA was extracted from the blood tissue according to the standard phenol and chloroform method [9].

Two pairs of oligonucleotide primers were synthesized to amplify two different fragments of 462 bp and 302 bp in length, based on available sequences of the bovine GHR gene exon 10 and 5' Non-coding region (Gene Bank accession number: AF 140284 and AB262325 respectively) using PRIMER-3 software (http://frodo.wi.mit.edu/). The primer sequences used for the 462-bp fragment containing a polymorphic *Alu*I restriction site at the 257 position were Forward Primer CAGCAGGAAATGTGGTCCTT; Reverse Primer GGGTAGCTCATGGGAAATCA, and for the 302-bp fragment containing a polymorphic *Nsi*I restriction site at the -154 position were Forward Primer CTGGCGTATGGTCTTTGTCA; Reverse Primer TGGTCTTGCTGCTTTCCTAT. The PCR was performed in a volume of 25µl using 1.5 mM $MgCl_2$, 200 µM dNTPs, 5 pmol of each primer, 50-100 ng of genomic DNA and 0.5 Units of Taq DNA polymerase (Bangalore Genei, India). Amplification reactions were carried with a program as 5 min denaturation at 94°C followed by 35 cycles of 94°C for 45 s, annealing at 55°C (462 bp) and 59°C (302bp) for 45 s and extension at 72°C for 1 min, with a final extension of 10 min at 72°C. For RFLP analysis, amplified product was digested with 1 unit of *Alu*I and *Nsi*I restriction enzymes (New England Biolabs) in a final volume of 20 µl. The amplified DNA was digested for overnight at 37^0C and separated by electrophoresis in 3% agarose in 1X TAE buffer for 90 minutes. The gel was stained with ethidium bromide, visualized under UV light, and photographed by Gel documentation system.

The genetic variability was evaluated in terms of the observed number of alleles (No) and observed Heterozygosity (Hobs) for each locus and a Hardy-Weinberg (H-W) equilibrium test was performed using POPGENE software [7].

Results and Discussion

PCR-*Alu*I Polymorphism at growth hormone receptor gene

At GHR locus, polymorphisms occurred at position 257 in Exon 10 (G/A substitution causing change of amino acid Ser to Gly) has been found to be associated with meat quality in Piemontese breed of catle. GHR^{A} variant has been reported to be associated with unfavourable effect on meat quality with higher drip losses (DL) [11]. The A allele at position 257 was also recognized by *Alu*I [16]. Rahbar *et al*. in 2010 [13] developed a PCR-based method for detecting *Alu*I RFLP in promoter region of the bovine growth hormone receptor (GHR) gene and it has been tested for association with milk-related traits in Holstein cows. They reported that allele frequency of the *Alu*I (-) and *Alu*I(+) alleles were 0.56 and 0.44, respectively. Polymorphic region of GHR exon 10 in 395 Polish Holstein-Friesian and 477 Polish Holstein-Friesian bulls was also screened with PCR-RFLP method giving the frequencies of alleles A are 0.832 and 0.891 and G are 0.168 and 0.109 for cows and bulls, respectively [10]. It is known to be a single nucleotide substitution (SNP), A/G (transition) recognizable by PCR-RFLP with *Alu*I.

In the present study, the PCR amplified DNA fragment of 462 bp was digested with *Alu*I restriction enzyme. *Alu*I restriction analysis of the PCR product yielded banding pattern corresponding to three different genotypes *viz*., AA genotype with one bands (93, 150, 191, 22, 6 bps), AG genotypes with three bands (93, 150, 191, 22, 6; 93, 100, 50, 191, 22, 6 bps) and GG genotype with two bands (93, 100, 50, 191, 22, 6 bps) as shown in Fig 1.

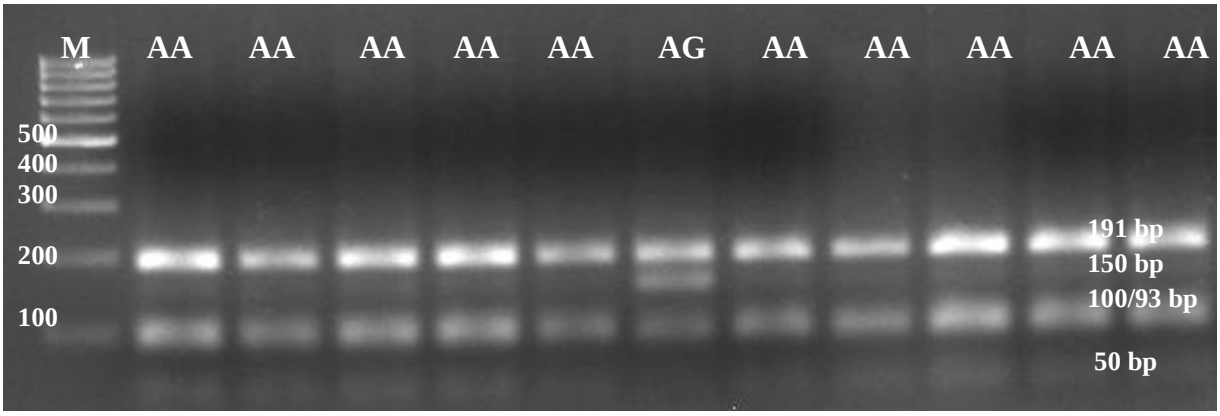

Fig. 1 Representative restriction pattern at GHR/*Alu*I locus on 3.0% agarose gel

The genotypic and gene frequencies at the GHR-*Alu*I locus in the investigated 10 breeds are presented in Table 1.

Table 1 No. of Animals, Observed Genotypes, Expected Genotypes, Genotypic frequency, Gene frequency and their χ^2 values of GHR-*Alu*I polymorphism in cattle

Breeds	No of Animals	Observed Genotypes			Expected Genotypes			Genotypic Frequency			Gene Frequency		
		AA	AG	GG	AA	AG	GG	AA	AG	GG	A	G	χ^2
Hariana	46	40	6	0	40.196	5.609	0.196	0.870	0.130	0.000	0.935	0.065	0.224
Kankrej	43	34	9	0	34.471	8.058	0.471	0.791	0.209	0.000	0.895	0.105	0.587
Mewati	43	38	5	0	38.145	4.709	0.145	0.884	0.116	0.000	0.942	0.058	0.164
Nagori	47	39	8	0	39.340	7.319	0.340	0.830	0.170	0.000	0.915	0.085	0.407
Tharparkar	47	44	3	0	44.048	2.904	0.048	0.936	0.064	0.000	0.968	0.032	0.051
Ghumusari	48	40	8	0	40.333	7.333	0.333	0.833	0.167	0.000	0.917	0.083	0.397
Hill Cattle	48	33	15	0	34.172	12.656	1.172	0.688	0.313	0.000	0.844	0.156	1.646
Kangayam	48	32	16	0	33.333	13.333	1.333	0.667	0.333	0.000	0.833	0.167	1.920
Binjharpuri	47	33	14	0	34.043	11.915	1.043	0.702	0.298	0.000	0.851	0.149	1.439
Punganur	36	26	10	0	26.694	8.611	0.694	0.722	0.278	0.000	0.861	0.139	0.937
Total	453	359	94	0	363.676	84.424	4.9	0.792	0.208	0.000	0.896	0.104	6.0463

Out of 453 animals screened 79.2% animals were homozygous for AA genotype and 20.8% of animals were heterozygous AG genotype. No homozygous GG animal was observed in any of the breed. The frequency distribution of A allele across the ten breeds ranged from 0.833 (Kangayam) to 0.968 (Tharparkar) with a mean value of 0.896, which was substantially high as compared to G allele in all the cattle populations, whereas that of G allele ranged from 0.032 (Tharparkar) to 0.167 (Kangayam) with a mean value of 0.104 (Table 1). This frequency is much higher than reported in Piomontese breed (0.49, in the group PTEST; 0.42, in the group MEAT) [11] and Angus calves (0.78) [16]. The observed and expected genotypic frequencies in each breed as well as the total population were again in Hardy-Weinberg Equilibrium ($p<0.05$) shown in Table 1.

GHR-*Nsi*I Polymorphism at –154 position in the 5' Non-coding region of growth hormone receptor gene

Several polymorphic sequences have been identified in the bovine GHR gene. Target regions of GHR gene were amplified and digested by *Alu*I, *Acc*I, *Stu*I, *Nsi*I, and *Fnu*4HI restriction enzymes has been found to be associated with milk and meat traits [8]. A single nucleotide polymorphism (A/G, transition) at GHR locus has also been reported at position -154 in 5' Non-coding region [2, 4]. The polymorphism was analyzed in ten cattle breeds by PCR-RFLP. A 302 bp DNA fragment

was amplified. *Nsi*I restriction analysis of the PCR product yielded banding pattern corresponding to three different genotypes *viz*., AA genotype with one bands (302 bp), AG genotypes with three bands (302, 167 + 135 bps) and GG genotype with two bands (167 + 135 bps) as shown in Fig 2.

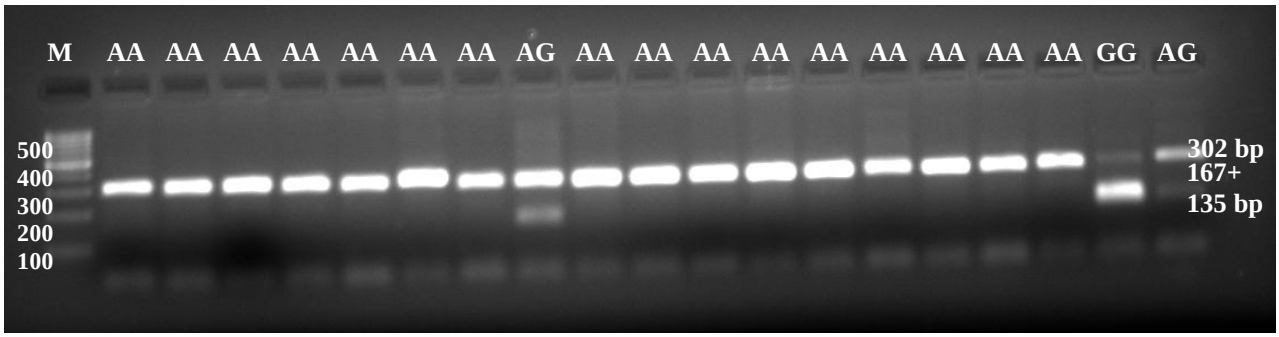

Fig. 2 Representative restriction pattern at GHR/*Nsi*I locus on 3.0% agarose gel

The genotypic and gene frequencies at the GHR-*Nsi*I locus in the investigated 10 breeds are presented in Table 2.

Table 2 No. of Animals, Observed Genotypes, Expected Genotypes, Genotypic frequency, Gene frequency and their χ^2 values of GHR-*Nsi*I polymorphism in cattle

Breeds	No of Animals	Observed Genotypes			Expected Genotypes			Genotypic Frequency (%)			Gene Frequency		
		AA	AG	GG	AA	AG	GG	AA	AG	GG	A	G	χ^2
Hariana	46	39	7	0	39.266	6.467	0.266	0.848	0.152	0.000	0.924	0.076	0.312
Kankrej	44	40	4	0	40.091	3.818	0.091	0.909	0.091	0.000	0.955	0.045	0.100
Mewati	43	41	2	0	41.023	1.953	0.023	0.953	0.047	0.000	0.977	0.023	0.024
Nagori	48	47	1	0	47.005	0.990	0.005	0.979	0.021	0.000	0.990	0.010	0.005
Tharparkar	45	45	0	0	45	0	0	1.000	0.000	0.000	1.000	0.000	0.000
Ghumusari	48	47	1	0	47.005	0.990	0.005	0.979	0.021	0.000	0.990	0.010	0.005
Hill Cattle	47	40	6	1	39.340	7.319	0.340	0.851	0.128	0.021	0.915	0.085	1.527
Kangayam	47	46	1	0	46.005	0.989	0.005	0.979	0.021	0.000	0.989	0.011	0.005
Binjharpuri	47	46	1	0	46.005	0.989	0.005	0.979	0.021	0.000	0.989	0.011	0.005
Punganur	36	34	2	0	34.028	1.944	0.028	0.944	0.056	0.000	0.972	0.028	0.029
Total	451	425	25	1	424.346	26.248	0.406	0.942	0.056	0.002	0.970	0.030	0.929

Out of 451 animals screened 94.2% animals were homozygous for AA genotype, 5.6% of animal were homozygous for GG genotype and 0.2% animals were heterozygous (AG genotype). The frequency of G allele was significantly lower than the A allele in all the cattle breeds. The frequency distribution of A allele across the ten breeds ranged from 0.915 (Tharparkar) to 1.00 (Hill Cattle) with a mean value of 0.970, whereas that of G allele ranged from 0.00 (Tharparkar)

to 0.085 (Hill Cattle) with a mean value of 0.30 (Table 2). This frequency is much higher than that reported (0.05) in Charolais, Limousin, Aberdeen Angus, Hereford meat cattle breeds [2] and lower than 0.61 in Polish Black and white dairy cattle [4]. The observed and expected genotypic frequencies in each breed as well as the total population were in Hardy-Weinberg Equilibrium ($p < 0.01$) as shown in Table 2. The study thus reveals that Indian zebu cattle have predominantly the desirable variant (A) at the investigated locus for superior milk quality and lean meat, which substantiates, at the genomic level, the general superiority of Indian cattle breeds in lean meat characteristics and higher milk constituents.

The difference in the taurine cattle and the Indian cattle is evident on the basis of allele frequency. One allele is almost fixed in taurine while the other allele is approaching fixation in Indian cattle.

Conclusion

Growth hormone receptor (bGHR) was investigated to characterize the variations at important gene loci and reveal their allelic status in the ten Indian cattle populations. Specific alleles at different gene loci (*e.g*., A allele at GHR-*Alu*I and *Nsi*I) were predominant and seems to be nearly fixed in Indigenous grey cattle breeds. Also their frequencies were in sharp contrast to that reported in exotic cattle. The reported genetic variant in the GHR-exon 10 was detected and frequency of undesirable variant GHR-A for meat was higher in all the indian cattle breeds investigated. The fixation of alleles at locus *viz*., *Nsi*I allele in naturally evolved ten *Bos indicus* populations having a higher milk constituents and lean meat quality than taurine breeds argues for the involvement of some additional alleles/genes influencing milk quality/production traits. Thus, there is need to investigate the Indian cattle with respect to various SNPs having associations with phenotypes of economically traits for their improvement.

Acknowledgement

We thank the Director, National Bureau of Animal Genetic Resources, Karnal and the Indian Council of Agricultural Research, New Delhi for providing all the necessary facilities in carrying out the present study.

References

1. A.J. Garrett, G. Rincon, J.F. Medrano, M.A. Elzo, G.A. Silver and M.G. Thomas, *J. Anim. Sci.*, Promoter region of the bovine growth hormone receptor gene: Single nucleotide

polymorphism discovery in cattle and association with performance in Brangus bulls, 2008, 86, 3315-3323.

2. A. Maj, L. Zwierzchowski, J. Oprzadek, A. Oprzadek and E. Dymnicki, *Anim. Res.*, Polymorphism in the 5'-noncoding region of the bovine growth hormone receptor gene and its association with meat production traits in cattle, 2004c, 53 (6), 503-514.
3. A. Maj, M. Korczak, J. Oprzadek, E. Zwierzchowski, E. Dymnicki, *Anim. Sci. Papers Report.*, TG repeat length polymorphism in the 5'-noncoding region of the growth hormone receptor gene in cattle and its association with meat production traits, 2004a, 22 (3), 297-305.
4. A. Maj, N. Strzalkowska, K. Sloniewski, J. Krzyzewski, J. Oprzadek and L. Zwierzchowski, *Czech*. *J. Anim. Sci.*, Single nucleotide polymorphism (SNP) in the 5′-noncoding region of the bovine growth hormone receptor gene and its association with dairy production traits in Polish Black-and-White cattle, 2004b, 49 (10), 419-429.
5. C.S. Hale, W.O. Herring, H. Shibuya, M.C. Lucy, D.B. Lubahn, D.H. Keisler and G.S. Johnsson, *J. Anim. Sci.*, Decreased growth in Angus steers with a short TG-microsatellite allele in the P1 promoter of the growth hormone receptor gene, 2000, 78, 2099-2104.
6. D.E. Moody, D. Pomp, W. Barendse and J.E. Womack, *Anim. Genet.*, Assignment of the growth hormone receptor gene to bovine chromosome 20 using linkage analysis and somatic cell mapping, 1995, 26, 341-343.
7. F. Yeh, C. Boyle, T. Rongcai, Z.Y. Ye and J.M. Xian, 1999, PopGene, Version 1.31. A Microsoft Windows-based free ware for population genetic analysis. University of Alberta, Edmonton. http://www.ualberta.ca/~fyeh/download.html.
8. Iraz Akis Akad, Ahmet Mengi, Kemal Ozdem Oztabak, *J. Vet. Anim. Sci.*, A determination of growth hormone receptor gene polymorphisms in East Anatolian Red cattle, South Anatolian Red cattle, and Turkish Grey cattle Turk, 2012, 36 (1), 27-33.
9. J. Sambrook and D. Russell, 2001, Molecular Cloning: A Laboratory Manual 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
10. K. Olenski, T. Suchocki and S. Kaminski, *Anim. Sci. Papers Report*., Inconsistency of associations between growth hormone receptor gene polymorphism and milk performance traits in Polish Holstein-Friesian cows and bulls, 2010, 28 (3), 229-234.

11. L. Di Stasio, G. Destefanis, A. Brugiapaglia, A. Albera and A. Rolando, *Anim. Genet.*, Polymorphism of the GHR gene in cattle and relationships with meat production and quality, 2005, 36, 138–140.
12. M. Falaki, N. Gengler, M. Sneyers, A. Prandi, S. Massart, A. Formigoni, A. Burny, D. Portetelle, R. Renaville, *J. Dairy Sci.*, Relationships of polymorphisms for growth hormone and growth hormone receptor genes with milk production traits for Italian Holstein-Friesian bulls, 1996, 79, 1446-1453.
13. R. Rahbar, G. Rahimi, Z. Ansari Pirsaraei and M. Gholizadeh, *Afr. J. Biotechnol.*, Identification of polymorphism in promoter region of growth hormone receptor (GHR) gene and its association with milk related traits in Holstein cows, 2010, 9 (33), 5460-5464.
14. S. Moisio, K. Elo, J. Kantanen and J. Vilkki, *Anim. Genet.*, Polymorphism within the 3'-flanking region of the bovine growth hormone receptor gene, 1998, 29, 55-57.
15. S.E. Aggrey, J. Yao, M.P. Sabour, C.Y. Lin, D. Zadworny, J.F. Hayes and U. Kunlein, *J. Hered.*, Markers within the regulatory region of the growth hormone receptor gene and their association with milk-related traits in Holsteins, 1999, 90, 148-151.
16. W. Ge, M.E. Davis, H.C. Hines and K.M. Irvin, *J. Anim. Sci.*, Rapid communication: Single nucleotide polymorphisms detected in exon 10 of the bovine growth hormone receptor gene, 2000, 78, 2229-2230.