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Association of growth hormone gene receptor polymorphism with production traits in Jamunapari goat

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ABSTRACT

Genetic polymorphism for GHR gene was carried out in Jamunapari kids by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Blood samples were collected from 200 kids for isolation of genomic DNA. The exon 1A (210 bp fragment), exon 10 (342 bp fragment) and 5' non-coding region (318 bp fragment) of GHR gene were amplified and digested with *MspI*, *AluI* and *NsiI* restriction endonuclease, respectively. PCR-RFLP analysis for 5' non-coding region revealed monomorphic pattern while exon 1A and exon 10 of GHR were polymorphic. The frequency of A and T alleles was 0.54 and 0.46, respectively, and frequency of three genotypes (AA, AT and TT) was 0.17, 0.74 and 0.09, respectively at locus exon 1A of GHR. The frequency of A and G allele for exon 10 was 0.53 and 0.47, and frequency of three genotypes (AA, AG and GG) was 0.16, 0.74 and 0.10, respectively. The genotype AG was associated with higher body weights at different growth stages. The goats with AG genotype were heavier to AA and GG genotypes by 4.91 and 42.85% at birth; 8.59 and 13.30% at 6 months; 8.02 and 17.25% at 9 months and 7.11 and 16.11% at 12 months, respectively. The locus was also a significant source of variation for biometrical traits (body weight, body length, heart girth) at different ages. AG genotype could be used as marker in selection for higher body weights in Jamunapari goats.

Key words: Growth hormone gene, Jamunapari goat, PCR-RFLP, Polymorphism, Production

Growth hormone (GH) is one of the major regulators of postnatal growth and metabolism in animals, and thus GH affects growth rate, body composition, health, milk production and aging (Bahrami *et al*. 2013). Growth hormone (GH) binds to its receptor (GHR) to which it has a high affinity for binding (Leung *et al*. 1987), causes receptor dimerization, and thus initiates signaling cascades through the cytoplasmic domain (Frank 2001), causing biological effects of GH on the target cells by the regulation of the transcription of the genes, including insulin-like growth factor-I (IGF-I), metabolic enzymes and transcription factors (Rotwein *et al.* 1994). GH receptor (GHR) consists of three parts including integrated membrane part, extracellular and intracellular domains. In addition to the membrane-bound growth hormone receptor (GHR), GH binding proteins (GHBPs) are associated with the extracellular domain of GHR (Leung *et al*. 1987 and Spencer *et al*. 1988).

GHR is a member of the cytokine/hematopoietin superfamily of receptors due to its partial amino acid homology and is a type I cytokine receptor. GHR utilizes the JAK (Janus kinase)/STAT (Signal transducers and activators of transcription) signalling pathway. However, GHR is also able to signal through additional pathways independent of JAK. In addition, GHR is targeted to the nucleus where it has important biological functions, especially with respect to cell proliferation (Brooks *et al*. 2008). The GHR gene is important candidate genetic marker for growth, carcass, and milk traits in livestock. Mutations in the GHR gene results in abnormalities which have been associated with Laron-type dwarfism in humans (Godowski *et al*. 1989), sex-linked dwarfism in chickens (Burnside *et al*. 1992), growth traits in beef cattle (Hale *et al*. 2000) and milk production traits in Holstein cattle (Aggrey *et al*. 1999). The present study aimed to reveal GHR gene polymorphism in Jamunapari goat breed reared at Central Institute for Research on Goats, Makhdoom and find association between GHR gene polymorphism and growth production traits in Jamunapari kids.

MATERIALS AND METHODS

Animal and data collection: A total of 200 blood samples were collected from Jamunapari kids maintained at livestock units of ICAR-Central Institute for Research on Goats (CIRG), Makhdoom, Mathura. The DNA was isolated from the blood samples using published standard protocol (Thangaraj *et al*. 2002). For association analysis of different variants with the growth production traits, data on biometrical traits of each doe was recorded on birth, 3, 6, 9 and 12 months age from the milk and growth registers.

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Table 1. Primers used for the amplification of GHR (exon 1, 10 and 5' UTR) gene

Gene/Locus	Primers (5'→3')	Annealing temperature (°C)	Size of amplicons (bp)
Exon 1	F GTGATTGGGAG GGAGGAAGAGA R CAAGGAGGGA GGGAGGAATAAAG	62	210
Exon 10	F GCTAACTTCATC GTGGACAAC R CTATGGCAT GATTTTGTTCAG	53	342
5 UTR	F CTGGCGTATGG TCTTTGTCA R TGGTCTTG CGCTTTCCT	66	318

DNA extraction and polymerase chain reaction: Amplification of GHR exon 1A, exon 10 and 5' noncoding region was done using set of primers (Maj *et al*. 2006, Di Stasio *et al*. 2005, Maj *et al*. 2004) (Table 1). The PCR reaction was performed in 50 µl final volume containing 1 µl of DNA template (100 ng), 1 µl of each primer (5 pmol), 1 µl of dNTPs (200 µM), 3 µl of $MgCl_2$ (25 mM), 4 µl of 10× assay buffer (Mg free-1×), 0.3 µl of Taq DNA Polymerase (Biolabs, 1 Unit) and 38.7 µl of autoclaved triple distilled water. Thermal cycling conditions for GHR exon 1A, exon 10 and 5' noncoding region were: 2 min at 97°C followed by 35 cycles of 94°C for 45 sec, 60°C for 45 sec and 72°C for 1 min, and finally extension of 10 min at 72°C.

PCR-RFLP: About 15 µl of the PCR product was digested with 10 U of *MspI, AluI* and *NsiI* endonucleases (New England Biolabs) for overnight at 37°C for GHR exon 1A (210 bp), exon 10 (342 bp) and 5' noncoding region (318 bp) respectively. All the components required for restriction enzyme digestion were prepared on ice. The resultant fragments of GHR exon 1A, exon 10 and 5' noncoding region were separated by electrophoresis in 2%, 3.5% and 2% agarose gels respectively, stained with ethidium bromide and analysed in a gel documentation system (Alpha Innotech Corporation, San Leandro, CA, USA).

Statistical analysis: The data on biometrical traits at birth, 3, 6, 9 and 12 months of ages were collected for Jamunapari goats from growth registers. Kids having incomplete/ abnormal (incurable disease) record of a trait were excluded from analysis. Data were analyzed using least-squares maximum likelihood programme (Harvey 1990). Genetic parameters, i.e. heritability (h^2) and genetic correlations (r_g) were estimated by paternal half sib correlation method. Effect of sire was estimated using mixed model incorporating sire as random effect, whereas period and season of birth, sex, type of birth, parity were estimated as fixed effect. Dams' weight at kidding was estimated by considering it as co-variable. Following statistical model was used to analyze the data:

$$Y_{ijklm} = \mu + P_i + S_j + R_k + T_l + U_m + b\,(A_{ijklm} - \hat{A}) + e_{ijklm}$$

Where, $Y_{ijklm,}$ body weight of kid born in i^{th} period, j^{th} season of birth, K^{th} sex, l^{th} type of birth, m^{th} parity; µ, population mean, P_i, effect of ith period of birth, S_j , effect of jth season of birth, R_k, effect of kth sex, T_l , effect of lth type of birth, U_m, effect of mth parity; b (A_{ijklm}), partial regression of Y_{ijklm} on body weight of kids on weight of dam at kidding; (A_{ijklm}), $\hat{A}$, average weight of doe at kidding and e_{ijklm} is residual random error associated with Y_{ijklm} and assumed to be identically and independently distributed with mean zero and constant variance.

Genepop (Raymond and Rousset 1995) software was used to estimate allelic frequencies, expected heterozygosity, effective number of alleles and to verify Hardy-Weinberg equilibrium. The genotypes were observed by counting the patterns in the gel documentation system. Variance and 95% confidence interval were calculated according to the formula (Var (p_u) = ½ N (p_u+P_{uu}–2p_u^2)) suggested by Weir (1996) (P_{uu}, genotypic frequency; p_u, allelic frequency).

RESULTS AND DISCUSSION

PCR-RFLP analysis of GHR exon1 and association with production traits: Genotyping of 200 kids was carried out with the *MspI* PCR-RFLP analysis. PCR analysis revealed an amplicon of 210 bp (Fig. 1). Three genotypes namely AA, AT and TT of (210 bp), (210 bp + 150 bp + 60 bp) and (150 bp + 60 bp) were obtained from the restriction digestion of amplified product (Fig. 2). The AT genotype was most frequently found with a frequency of 0.74 whereas frequency of AA and AT genotype was moderate (0.17) and low (0.09) in these kids (Table 2). The allelic frequency of A and T was 0.54 and 0.46, respectively (Table 2). *MspI* digestion of GHR exon 1 region revealed that heterozygous AT genotype was most frequent as compared to homozygous AA and TT in Jamunapari kids.

PCR-RFLP analysis of exon 1with *MspI* restriction

Table 2. Genotypic and allelic frequencies of GHR (exon 1 and 10) in Jamunapari kids

	Exon 1					Exon 10				
	Genotypic frequency			Allelic frequency		Genotypic frequency			Allelic frequency	
Variable	AA	AT	TT	A	T	AA	AG	GG	A	G
Number	34	148	18	108	92	32	148	20	106	94
Frequency	0.17	0.74	0.09	0.54	0.46	0.16	0.74	0.09	0.53	0.47

Table 3. Least square means of different biometrical traits at different ages of various genotypes of GHR gene in Jamunapari kids

Effect	BBL	BBH	BHG	3BL	3BH	3HG	6BL	6BH	6HG (In inch)
Exon 1				**	**	**	**	**	
AA	33.8±0.4 (34)	35.2±0.4 (34)	33.1±0.4 (34)	46.8±0.6 (34)	47.6±0.6 (34)	45.7±0.5 (34)	52.8±0.7 (33)	55.0±0.6 (33)	52.7±0.7 (33)
AT	33.2±0.2 (148)	34.7±0.23 (148)	33.1±0.2 (148)	48.0±0.3 (142)	48.8±0.3 (142)	46.6±0.3 (142)	55.2±0.4 (135)	56.2±0.3 (135)	53.8±0.4 (135)
TT	32.3±0.6 (18)	34.3±0.5 (18)	32.6±0.6 (18)	44.8±0.9 (16)	45.1±0.9 (16)	43.7±0.8 (16)	49.8±1.0 (15)	53.6±1.0 (15)	51.7±1.0 (15)
Exon 10	**	**	**				**	**	**
AA	31.7±0.5 (32)	33.5±0.6 (32)	30.1±0.5 (32)	48.8±0.7 (32)	50.2±0.4 (32)	47.9±0.4 (32)	54.0±0.6 (32)	55.5±0.5 (32)	53.4±0.5 (32)
AG	32.3±0.5 (148)	34.2±0.5 (148)	30.9±0.5 (148)	49.5±0.4 (144)	49.8±0.3 (144)	47.7±0.3 (144)	55.0±0.3 (140)	55.7±0.3 (140)	53.3±0.3 (140)
GG	30.1±0.7 (20)	32.1±0.7 (20)	29.0±0.7 (20)	48.7±1.1 (20)	49.9±0.7 (20)	47.8±0.7 (20)	51.3±0.9 (20)	52.0±0.8 (20)	50.9±0.7 (20)

*Significant (P<0.05), **Significant (P< 0.01). BBL, Body length at birth; BBH, body height at birth; BHG, heart girth at birth; 3BL, body length at 3 month; 3BH, body height at 3 month; 3HG, heart girth at 3 month; 6BL, body length at 6 month; 6BH, body height at 6 month; 6HG, heart girth at 6 month.

Effect	9BL	9BH	9HG	12BL	12BH	12HG (In inch)
GHR Exon 1	**	**	**	**	**	**
AA	60.0±0.8 (33)	61.0±0.7 (33)	58.7±0.7 (33)	64.1±0.8 (29)	66.1±0.8 (29)	63.7±0.8 (29)
AT	62.5±0.4 (135)	63.2±0.4 (135)	60.7±0.4 (135)	66.8±0.4 (128)	67.9±0.4 (128)	65.5±0.4 (128)
TT	58.0±1.1 (15)	59.6±1.0 (15)	56.6±1.0 (15)	62.2±1.2 (15)	64.6±1.2 (15)	61.7±1.2 (15)
GHR Exon 10	*	*		**	**	**
AA	61.0±0.5 (32)	61.8±0.5 (32)	59.4±0.5 (32)	67.6±0.5 (32)	68.9±0.6 (32)	66.2±0.6 (32)
AG	62.5±0.3 (136)	63.0±0.3 (136)	60.8±0.3 (136)	67.7±0.3 (126)	69.0±0.4 (126)	66.2±0.3 (126)
GG	62.9±0.8 (20)	64.2±0.8 (20)	61.2±0.8 (20)	61.8±0.8 (20)	63.6±0.9 (20)	61.1±0.8 (20)

*Significant (P<0.05), **Significant (P<0.01). 9BL, Body length at 9 month; 9BH, body height at 9 month; 9HG, heart girth at 9 month; 12BL, body length at 12 month; 12BH, body height at 12 month; 12HG, heart girth at 12 month.

enzyme indicated the presence of A and T alleles in Jamunapari goat breed which were in agreement with the results of Maj *et al*. (2006). They reported C and T alleles in five sheep breeds. A 317 bp long interspersed repetitive element (LINE-1) fragment located 482 bp upstream of the transcription start site for exon 1A was amplified in goats (Maj *et al*. 2005). Lucy *et al*. (1988) identified a 1206 bp retrotransposon, a LINE-1 element, located within the P1 promoter for exon 1A in the bovine GHR gene. Aggrey *et al*. (1999) identified three RFLPs in the P1 promoter region of the bovine GHR gene, recognized with restriction endonucleases *AluI*, *AccI*, and *StuI*. Seven SNPs were found within the coding part of the gene and in the introns by Blott *et al*. (2003).

Exon 1A locus of GHR gene was a significant source of variation for the body weights at different ages (Table 3). The animals with genotype AT were higher in their body weights in comparison to the animals with genotypes AA and TT (Table 3). Similarly, the locus significantly influenced all biometrical traits except at birth (body length, body height and heart girth) and heart girth at 6 months (Table 4). Animals with AT genotype were superior to AA and TT genotype for all biometrical traits in the flock of Jamunapari goats. The corresponding superiority of AT over TT was 10.72 (body length) and 4.90% (body height) at 6 months, while for other biometrical traits AT genotypes were superior over AA and TT (Table 4). The superiority of AT for body length, height and girth as compared to AA was 2.47, 2.39 and 1.95% at 3 months; 4.16, 3.60 and 3.36% at 9 months and 4.27, 2.73 and 2.69% at 12 months of age, respectively. The corresponding superiority of AT over TT was 7.08, 8.19 and 6.69% at 3 months; 7.87, 6.09 and 7.21% at 9 months and 7.33, 5.13 and 6.17% at 12 months of age, respectively.

PCR-RFLP analysis of GHR exon 10 and association with production traits: Genotyping of exon 10 of GHR gene was carried out in 200 Jamunapari kids for the presence of different alleles using *AluI* PCR-RFLP method. The amplified PCR product was observed as 342 bp (Fig. 3). *AluI* restriction pattern revealed three genotypes of GHR exon 10, i.e. AA (342 bp), AG (342 bp + 292 bp + 50 bp) and GG (292 bp + 50 bp) (Fig. 4). The frequencies of AA, AG and GG genotypes were 0.16, 0.74 and 0.09, respectively in kids (Table 2).

PCR-RFLP analysis with *AluI* restriction enzyme revealed three genotypes of GHR exon 10 i.e. AA, AG and

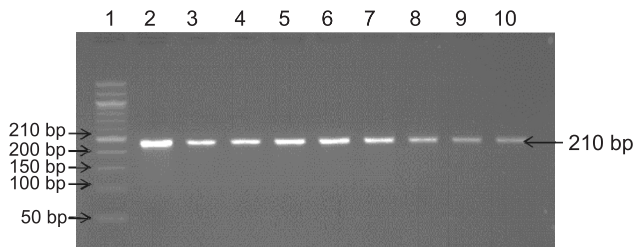

Fig 1. PCR amplification of exon 1A region of GHR gene in Jamunapari kids. Lane 1, Marker (50 bp); lanes 2–10, amplified PCR products.

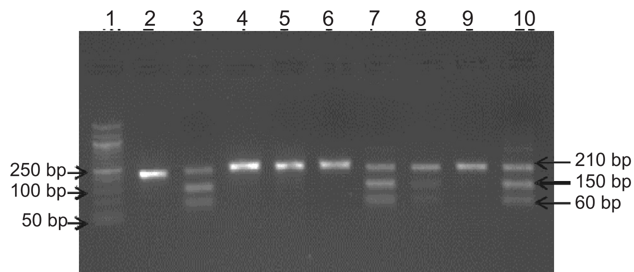

Fig. 2. *MspI*-RFLP genotype of exon 1 region of GHR gene in Jamunapari kids. Lane 1, Marker (50 bp); lane 2, original PCR product; lanes 3–9, digested PCR products.

GG. The frequency GHR^A allele was high (0.53) in Jamunapari kids (Table 2). Present findings were in agreement with those reported by Aggrey *et al*. (1999), Maj *et al*. (2010, 2006 and 2004), Vitala *et al*. (2006) and Hradecka *et al*. (2008). Two polymorphic sites of the exon 10 of Qinchuan cattle and their positive association with growth traits were reported by Zhao *et al*. (2007), suggesting the possibility of using GHR gene as a candidate gene for growth traits in Quinchuan cattle. Alsiding *et al*. (2010) reported polymorphisms in exon10 of GHR gene of Baggara cattle and explained a number of variations between Baggara types and Angus cattle GHR gene which were responsible for transducing the myogenic signal of growth hormone across the cell membrane.

The locus exon 10 significantly influenced the body weights at different ages except 3 months weight (Table 3). The heterozygous animals with AG genotype were heavier to the homozygous animals with AA and GG genotypes by 4.91 and 42.85% at birth; 8.59 and 13.30% at 6 months; 8.02 and 17.25% at 9 months and 7.11 and 16.11% at 12 months, respectively. Similarly, the locus significantly influenced all biometrical traits except those at 3 months and hind girth at 9 months (Table 4). AG genotype had better body biometrical traits than AA and GG genotypes. The superiority of AG for body length, body height and heart girth as compared to AA and GG genotypes was 1.92, 1.87 and 2.78% and 7.12, 6.01 and 2.89%, respectively at birth. The corresponding AG genotype superiority were 0.05, 0.11 and 0.06% and 9.57, 8.47 and 8.30%, respectively, at age of 12 month. The corresponding superiority of AG over AA and GG was 1.86 and 0.43% and 7.30 and 7.18%, for body length and body height at 6 months, respectively while

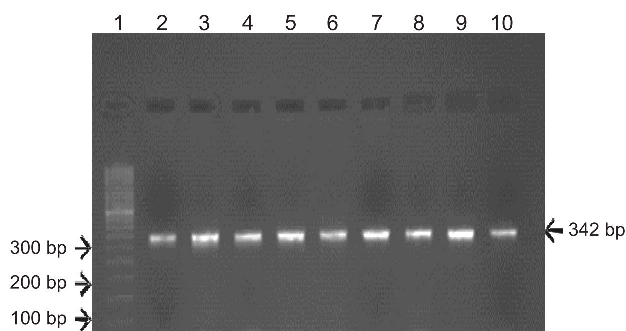

Fig. 3. PCR amplification of exon 10 of GHR gene in Jamunapari kids. Lane 1, Marker (50 bp); lanes 2–10, amplified PCR products.

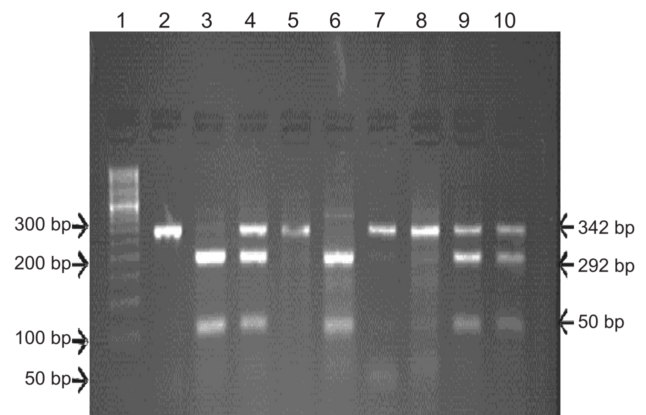

Fig. 4. *AluI* genotyping of exon 10 of GHR gene in Jamunapari kids. Lane 1, Marker (50 bp), lane 2, original PCR product; lanes 3–10, digested PCR products.

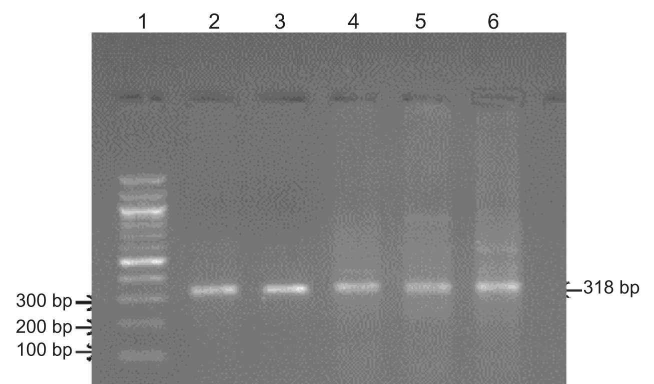

Fig. 5. PCR amplification of 5' noncoding region of GHR gene in Jamunapari goats. Lane 1, Marker (100 bp); lanes 2–6, amplified PCR products.

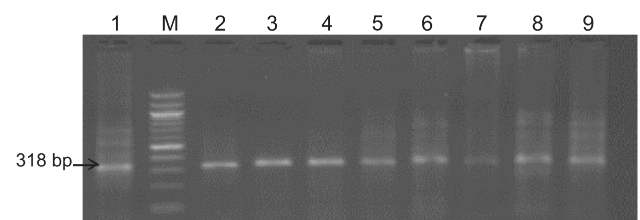

Fig. 6. *NsiI*-RFLP genotype of 5’ noncoding region of GHR gene in Jamunapari goats. Lane 1, Original PCR product; lane M, marker (100 bp); lanes 2–9, digested PCR products.

for heart girth at 6 months, AA was 0.22% superior than AG and 4.65% superior than GG. For 9 month weight, GG was superior over AA and AG for body length by 0.65 and 3.06% and for body height by 3.84 and 1.79%, respectively.

PCR-RFLP analysis of GHR 5' noncoding region: 5' noncoding region of GHR was genotyped by PCR-RFLP method in 200 Jamunapari kids. PCR amplified product was observed as 318 bp (Fig. 5). The PCR product of 318 bp was digested with *NsiI* restriction enzyme. The RFLP analysis showed only AA (318 bp) genotype in Jamunapari goats (Fig. 6) so it was considered as monomorphic. However, it cannot be concluded by the absence of polymorphic sites in this region that the whole gene is not polymorphic. Absence of any mutation in the 5'-noncoding region of GHR gene in Jamunapari goats indicated its highly conserved nature. However, larger population of animals needs to be screened for better understanding of variability of 5'-noncoding region of GHR gene in Jamunapari goats.

However, Maj *et al*. (2004) reported *NsiI* polymorphic sites in Polish Black and White cattle and correlated with dairy production traits. In another study, Maj *et al*. (2006) reported polymorphism in the 5'-noncoding region of Polish Black and White cattle and its positive association with meat production traits.

In summary, our present results showed associations between genetic variants at the exonic region of the caprine GHR gene, and growth production traits and other traits related to meat production, including for biometrical traits (body weight, body length, heart girth) and body weight at different stages (birth, 3, 6, 9, 12 month body weight). However, to draw final conclusions, these studies should be extended to a larger and more homogenous population of goat, preferably with the use of a reference family.

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