Characterization of sequence variation in caprine growth hormone gene and its association with milk production traits in two Indian goat breeds

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ABSTRACT

Genetic polymorphism in complete caprine growth hormone (*GH*) gene was investigated by screening 309 DNA samples of both sexes belonging to two Indian goat breeds namely *Jamunapari* (140) and *Sirohi* (169) using PCR - single strand conformation polymorphism (SSCP) and DNA sequencing. Twenty four SNPs located in promoter (C160A G164A, T193C), intron 1 (C400T, A497G, G499A and G563A), exon 2 (G781A), exon 3 (G1121A, C1148T, G1170A), intron 3 (G1212T, G1284C), exon 4 (G1442A, A1532G, G1551A, G1578C and C1585T), intron 4 (G1602C), exon 5 (G1938C, G1956C, G2049C, G2058C) and 3 UTR (T2197A) regions were identified. All the identified SNPs were analyzed for association with different milk production traits. Out of all polymorphic loci analyzed, the SNP located at 781th bp of GH had significant effect on different milk production traits of Sirohi goats. Locus GH_164 and GH_1212 of GH gene were found to be significantly associated with milk production traits in Jamunapari goats. The SNPs at the caprine GH associated with the milk production traits may be used as markers in selection of goats for high milk yield.

Keywords: Markers, GH gene, SNP, Jamunapari, Sirohi

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INTRODUCTION

Production traits of domestic animals are always of major concern in livestock especially in breeding for its determinant economic value. Growth hormone (GH) gene is the main regulator of milk production, postnatal somatic growth, stimulating anabolic processes such as cell division, skeletal growth and protein synthesis (Katoh et al. 2008). Therefore, it has been widely used as candidate marker in several livestock species including cattle (Katoh et al. 2008), sheep (Marques et al. 2006), and goat (Malveiro et al. 2001; Hua et al. 2009; Wickramaratne et al. 2010; Zhang et al. 2011). Most of the studies on *GH* gene polymorphisms and their association with production traits have been conducted mainly in Cattle. There are few reports on polymorphisms in entire GH gene and their association with traits of economic importance in goats. Hence, the objectives of present study were to identify single nucleotide polymorphisms (SNPs) in entire *GH* gene and to determine their influence on milk production traits of *Jamunapari* and *Sirohi* goats.

MATERIALS AND METHODS

Animals and phenotypic data

The data on *Jamunapari* (N=140) and *Sirohi* (N=169) goats were collected from the flocks maintained at Central Institute for Research on Goats (CIRG), Makhdoom, Mathura (U.P., India) and Livestock Research Center, Vallabhnagar (Rajasthan). Milk yield data at an interval of 90 and 140 days was collected. The protein, fat and solid not fat percentage of milk was estimated using electronic milk tester namely lactoscan (Bulgaria made).

Blood samples collection and DNA extraction

Approximately 8-10 ml blood was collected from each animal in EDTA coated vacutainer tubes and stored in deep freezer at -20°C till DNA isolation. Genomic DNA was isolated using standard procedure of proteinase-K digestion (Sambrook et al. 1989). After checking the quality and quantity of genomic DNA, it was diluted to a final concentration of 50-100 ng /µl in nuclease free water and stored at 4°C for further use.

Table 1. Fragment location, primer sequence and annealing temperature & details of primers used in amplifying growth hormone gene

Location	Primer sequence (5'-3')	Amplicon Size (bp)	Annealing Temp (^{0}C)
Promoter 5' (13-363 bp)	F - cccagggattaaacctgagtc R - ctctgctgggccctttttat	325	63.0
5'-U-E1-I1(345-693 bp)	F - gggggaaagggagagagaag R - ccctagggagagaccaggag	379	64.0
I1-E2-I2 (674-1030 bp)	F – gatcaggcatccagctctct R – tcactgccttattcggaacc	396	60.0
I2-E3-I3 (1011-1344 bp)	F - ggttccgaataaggcagtga R - caccaccaccaaccatcat	449	63.5
I3-E4-I4 (1326-1667 bp)	F - ccaccaaccacccatctgcc R - gaagggaccaagaacgcc	214	60.0
I4-E5-D(1648-2012 bp)	F - ctagcagccagtcttgacc R - ggggaggggtaacaacagat	388	56.0
E5-3'-D(1993-2344 bp)	F - ctgcacaagacggagacgta R - tcacagagaaggggatgtgc	352	58.0
3'-D (2325-2506 bp)	F - gcacatccccttctctgtga R - cttcccactcttggaggcta	182	56.0

U = up stream E = exon, I = intron, D = downstream, bp = base pairs, F – Forward primer, R – Reverse primer

Primer synthesis and PCR amplification

Primers were designed based on published goat *GH* sequence (accession number D00476) by PRIMER3 software available online (http://frodo.wi.mit.edu/cgibin/primer3/primer3_www_slow.cgi). Eight sets of overlapping primers at 5' and 3' ends were designed to amplify the complete GH gene sequence in the studied goat breeds (Table 1). Polymerase chain reaction was carried out in a final reaction volume of 25 µl in thermocycler (PTC-thermal cycler, MJ research, USA). The annealing temperature of all the fragments ranged from 56-64˚C. The amplified product was analyzed by electrophoresis on 1.5% agarose gel at 100V for 20 minutes using ethidium bromide staining.

Single strand conformation polymorphism (SSCP) and DNA sequencing

SSCP analysis was carried out as per standard protocol (Orita et al. 1989). The samples showing unique band pattern in SSCP gels for each investigated fragment were selected for further DNA sequencing. The PCR product of 10 µl of each unique sample was purified with PCR purification kit (Labmate) and was sequenced with ABI 3100 automated DNA sequencer (Applied Biosystem). The "Edit seq"of DNA star software was used for formatting the sequences to make them compatible with the other desired softwares. The sequences were aligned with respect to the complete goat reference sequence (Kioka et al. 1989) downloaded from NCBI by using Clustal W software (Thompson et al. 1997). The sequence data were then analyzed with Chromas Pro 1.49 (http:/www.technelysium.com.au) and Blast 2.0 (Altschul et al. 1990) softwares for identification of SNPs.

Statistical analysis

Association between polymorphism of GH gene and milk traits was analyzed using general linear model of SPSS software (Version 8.0). The following model was used to analyse the significant effect of genotype of GH gene on milk traits.

$$Y_{ijklmn} = \mu + S_i + T_j + O_k + G_l + P_m + b\,(x_{ijklm} - x) + e_{ijklmn}$$

Y_{ijklmn} = Milk traits of the n^{th} individual born in i^{th} season, j^{th} kidding type, k^{th} kidding order, l^{th} genotype and with m^{th} sex.

x_{ijklm}= Weight of doe of n^{th} individual in i^{th} season, j^{th} kidding type, k^{th} kidding order, l^{th} genotype and with m^{th} sex

μ = Overall mean

S_i = Fixed effects of i^{th} seasons

$T_{j=}$ Fixed effects of j^{th} kidding type

O_k = Fixed effect of k^{th} kidding order

G_l = Fixed effect of l^{th} genotype

P_m = Fixed effect of m_{th} sex

b = Regression coefficient of milk traits on doe's weight at kidding

RESULTS AND DISCUSSION

Polymorphisms of GH gene

The complete growth hormone gene was amplified using eight overlapping fragments. SSCP analysis revealed 1 to 5 distinct variants across the different fragments. The polymorphism observed in both the breeds has been presented in Table 2. A total of twenty four SNPs located in promoter (C160A G164A, T193C), intron 1 (C400T, A497G, G499A and G563A), exon 2 (G781A), exon 3 (G1121A, C1148T, G1170A), intron 3 (G1212T, G1284C), exon 4 (G1442A, A1532G, G1551A, G1578C and C1585T), intron 4 (G1602C), exon 5 (G1938C, G1956C, G2049C, G2058C) and 3 UTR (T2197A) regions were identified. To our best knowledge, all the SNPs except four ones at 781, 1148, 1532 and 1585bp are novel ones in Indian goats. In our earlier study on Osmanabadi and Sangamneri breeds of goat (Wickramaratne et al. 2010), SNPs at 164, 193, 497, 499, 1551, 1578 and 1585bp of GH gene were also detected (Wickramaratne et al. 2010). Jamunapari goats were monomorphic at the promoter (160 bp, 164bp), intron 1 (400bp) while *Sirohi* breed was observed monomorphic at intron 1 (497bp, 499bp, 564bp) and exon 3 regions (1121-1170bp). Our results are consistent with polymorphism at 781, 1148, 1532 and 1585bp reported in other flocks such as Chengdu-Ma, Boer, Savanna and Kalahari goats (Li et al. 2004, Hua et al. 2009, Amie Marini et al. 2012). The exon 1 region in the present study was homozygous and monomorphic which was consistent with the reports on other Indian goat breeds (Wickramaratne et al. 2010) as well as in sheep (Ofir R & Gootwine E 1997).

Effect of GH genotypes on milk production traits

Three out of twenty four investigated SNPs influenced the milk traits of *Jamunapari* and one of twenty four in the *Sirohi* goats (Table 3, 4). Locus G164A significantly influenced the milk yield at 90, 140 days and total milk yield and effect was non-significant on rest of the studied traits in *Jamunapari* goats (Table 4). The locus explained 3.7% (Milk yield at 140 days) to 4.2% (milk yield at 90 days) of the phenotypic variability for milk. The animals with genotype GA yielded more milk compared to the animals with genotype GG by 18.99, 17.83 and 18.21% at 90, 140 days and total milk yield, respectively. Locus G164A of *Sirohi* breed was polymorphic but no pattern was significantly associated with any of the traits (Table 4).

Table 2. The location and polymorphism detected in growth hormone (GH) gene of Sirohi and Jamunapari breeds of goats

Fragment	Region	Nucleotide Position	Sequence change	Sequence[2]	Reference sequence	Breeds		Remark[2]	Amino Acid change/ codon number
						Sirohi	Jamunapari		
5'UTR (13-364)	5'UTR	160	C/A	GCCATCA	GCCCTCA	+	-	Tv	
		164	G/A	TCAAGCT	TCAGGCT	+	+	Ts	
		193	T/C	TGACGAG	TGATGAG	+	+	Ts	
E1- I1 (315-693)	Intron1	400	C/T	AGATGAC	AGACGAC	+	-	Ts	
		497	A/G	GGGGGAA	GGGAGAA	-	+	Ts	
		499	G/A	GAGAACT	GAGGACT	-	+	Ts	
		564	G/A	TCTACAC	TCTGCAC	-	+	Ts	
I1-E2-I2 (635-1030)	Exon 2	781	G/A	TCCAGCC	TCCGGCC	+	+	Ts	Ser-Gly (S-G)/35

I2-E3-I3 (1011-1344)	Exon 3	1121	G/A	CCAAAAC	CCAGAAC	-	+	Ts	No Change AA / 72
		1148	C/T	CTCTGAA	CTCCGAA	-	+	Ts	No Change AA / 81
		1170	G/A	ACGAGCA	ACGGGCA	-	+	Ts	Gly-Ser G-S / 89
	Intron 3	1212	G/T	CTATGAC	CTAGGAC	+	+	Tv	
		1284	G/C	GGCCTTC	GGCGTTC	+	+	Tv	
I3-E4-I4 (1318-1754)	Exon 4	1442	G/A	TTCACAT	TTCGCAT	+	+	Ts	
Arg-His (R-H) / 103		1532	A/G	CGGGCCG	CGGACCG	+	+	Ts	
Asp-Gly (D-G) / 133		1551	G/A	GCTAAAG	GCTGAAG	+	+	Ts	No Change AA / 139
		1578	G/C	GGCCCTG	GGCGCTG	+	+	Tv	No Change AA/148
		1585	C/T	ATGTGGG	ATGCGGG	+	+	Ts	Arg-Try (R-W) / 151
	Intron 4	1602	G/C	GTTCTTG	GTTGTTG	+	+	Tv	
I4-E5-I5 (1706-2093	Intron 4	1831	-/C	GCCCTTC	GCC-TTC	+	+	In	Insertion
	Exon 5	1938	G/C	GCGCAGT	GCGGAGT	+	+	Tv	No Change AA / 176
		1956	G/C	GCTCAAG	GCTGAAG	+	+	Tv]	No Change AA / 182
		2049	G/C	GGCCAGC	GGCGAGC	+	+	Tv	No Change AA/213
		2058	G/C	CGCCTTC	CGCGTTC	+	+	Tv	No Change AA / 216
E5-3'UTR (1993-2344)	3 UTR	2088	C/-	ACCC-TCC	ACCCCTCC	+	+	Del	Deletion
		2197	T/A	TTCAAGG	TTCTAGG	+	+	Tv	
Downstream 3' (2325-2506)	3down region stream		Monomorphic			+	+		

a Nucleotide change observed(bold) when aligned with exotic sequence (Accession No. D00476), no nucleotide changes within the population in both the breeds.
b (+)Nucleotide variation detected in that breed, (-) Not detected; c AA-Amino acid, Gly-Glycine, Ser- Serine, Arg- Arginine, His- Histine, Asp-Aspartate, Try- Tyrosine ; d Ts : Transition, Tv: Transversion

Table.3: Least-square analysis of different milk production traits of various genotypes of GH gene in Jamunapari goats

Position	Fragment	Genotypes(N)	Fat (%)	Protein (%)	Lactose (%)	SNF (%)	90DMY (l)	140DMY (l)	TMY (l)	LL (days)
GH_164										
G/A	5'UTR		N.S	N.S	N.S	N.S	S	S	S	N.S
		GA (103)	3.58±0.32	3.46±0.06	5.09±0.09	9.40±0.18	84.21±4.08a	123.07±5.69a	148.52±7.15a	198.43±3.86
		GG (37)	3.87±0.38	3.36±0.07	4.95±0.11	9.10±0.21	70.77±4.90b	104.44±6.83b	125.64±8.58b	197.74±4.64
		P value					0.038	0.039	0.043	
		R 2 Value								
				4.26	3.70	3.18				
GH_1148										
C/T	Exon 3		N.S	N.S	N.S	N.S	N.S	S	S	N.S
		CT (55)	2.71±0.53	3.47±0.10	5.11±0.16	9.42±0.30	92.16±7.31	141.33±10.19a	177.11±12.69a	201.60±6.63
		GT	3.15±0.49	3.42±0.05	5.07±0.15	9.34±0.28	94.17±6.77	139.23±9.44b	172.56±11.75b	208.11±6.14
		TT (62)	3.73±0.26	3.42±0.05	5.04±0.08	9.30±0.15	78.62±3.58	115.68±5.00b	138.60±6.22b	195.68±3.25
		P value						0.019	0.004	
		R 2 Value								
					8.75	11.35				
GH_1212	G/T	Intron 3	N.S	N.S	N.S	N.S	S	S	S	N.S
		GG (45)	2.94±0.33	3.45±0.07	5.11±0.11	9.41±0.20	94.30±4.96a	141.85±6.86a	177.51±8.81a	206.14±4.56
		TT (55)	3.40±0.27	3.45±0.05	5.09±0.09	9.39±0.16	78.03±3.98b	116.41±5.51b	141.45±7.07ab	195.90±3.66
		GT (40)	4.57±0.68	3.30±0.15	4.81±0.22	8.89±0.42	89.16±10.07b	127.55±13.94b	137.76±7.88b	199.83±9.26
		P value					0.039	0.018	0.005	
		R 2 Value					7.42	8.83	11.50	

Values with different superscripts in the same column are significantly different at P≤ 0.05, N.S.= Non-significant, S-Significant, 90DMY = 90 Days milk yield, 140DMY = 140 Days milk yield, TMY = Total milk yield, LL = Lactation length, N = Number of observation, SNF = Solid not fat, UTR= Untranslated region, (l)-liter

Table 4.: Least-square analysis of different milk production traits of various genotypes of GH gene in Sirohi goats

SNP	Fragment	Genotype (N)	Fat (%)	Protein (%)	Lactose (%)	SNF (%)	LL	TMY
	Exon 2		N.S.	N.S.	**S**	**S**	N.S.	N.S.
G781A		GG (135)	5.44±0.91	3.27±0.08	4.75±0.12a	8.76±0.22a	152.81±0.91	95.91±2.66
		AA (34)	4.23±1.10	3.52±0.10	5.15±0.15b	9.48±0.27a	153.44±1.11	96.31±3.23
	P value				0.048	0.046		
	R 2 Value				4.56	4.66		

Values with different superscript in the same column are significantly different at P ≤0.05, **NS**- Non-significant, **S**- Significant, N- Total number of animals with respective genotype, **SNF-**Solid not fat, **TMY-** Total milk yield, **LL-** Lactation length

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CONCLUSIONS

The SNPs (G164A, G781A, C1148T, G1212T,) at the caprine GH that has been found to be associated with the milk production traits may be used as markers in selection of goats for superior milk quality and milk yield. However, as the study was based on a limited sample size, reconfirmation of findings is warranted with an adequate sample size. The nucleotide changes which were commonly detected in both the studied breeds need to be investigated in other Indian goat breeds as well to confirm whether they are specific only to Indian goat breeds. Further, the SNPs detected at the caprine GH gene might provide useful information for the phylogeny of different goat breeds.

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ. 1990. Basic local alignment search tool.

Journal of Molecular Biology 215: 403-410.

Amie Marini AB, Aslinda K, Moh. Hifzan R, Muhd Faisal AB and Musaddin K. 2012. HaeIII-RFLP Polymorphism of growth hormone gene in Savanna and Kalahari goats. Malaysian Journal of Animal Science 15:13-19.

Beckmann JS, Kashi Y, Hallerman FM, Nave A and Soller M. 1986. Restriction Fragment Length Polymorphism among Israeli Holstein-Friesian dairy bulls *Animal. Genetics* **17**: 25-38.

Chitra R and Aravindakshan TV. 2004. Polymorphism at growth hormone gene in *Malabari* goats investigated by PCR-RFLP. *Indian Journal of Animal Sciences* 74 (12): 1215-1218.

Cowan CM, Dentine MR. and Schuler LA. 1989. RFLP associated with growth hormone and prolactin gene in Holstein bulls: evidence for a novel growth hormone allele. *Animal Genet*ics 20: 157-165.

Falaki M, Prandi A, Corradini C, Sneyers M, Gengler N, Massart S, Fazzini U, Burney A., Portetelle, D. and Renaville, R. 1997. Relationship of growth hormone gene and milk protein polymorphism to milk production traits in Simmental cattle. *Journal of Dairy Research* 64: 47-56.

Hallerman EM, Nave A, Kashi Y, Holzer Z and Beckman JS. 1987. Restriction fragment length polymorphisms in dairy and beef cattle at the growth hormone and prolactin loci. *Animal Genet*ics. 18: 213-222.

Hillbert P, Marcotte A, Schwers A, Hanset R, Vassart G and Georges M. 1989. Analysis of genetic variation in the Belgian Blue Cattle breed using DNA sequence polymorphism at the growth hormone, low density lipoprotein receptor, α-subunit of glycoprotein hormones and thyroglobulin loci. *Animal Genet*ics 20**:** 383.

Hua GH, Chen SL, Yu JN, Cai KL, Wu CJ, Li QL, Zhang CY, Liang AX, Han L, Geng LY, Shen Z, Xu DQ and Yang LG. 2009. Polymorphism of the growth hormone gene and its association with growth traits in Boer goat bucks. *Meat Science* 81(2): 391–395.

Katoh K, Kouno S, Okazaki A, Suzuki K and Obara Y. 2008. Interaction of GH polymorphism with body weight and endocrine functions in Japanese black calves. *Domestic Animal Endocrinology* 34(1): 25–30.

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 Biological Chemistry* 53: 1583–1587.

Li MY, Pan QH, Pan YC, Shen W, Min LJ and Ren ZY. 2004. Polymorphism analysis of goat growth hormone (GH) gene by PCR-RFLP. *Journal of Laiyang Agricultural Colleague* 21(1), 6–9 (in Chinese, with English abstract).

Malveiro E, Pereira M, Marques PX, Santos IC, Belo C and Renaville R. 2001. Polymorphisms at the five exons of the growth hormone gene in the algarvia goat: Possible association with milk traits. *Small Ruminant Research.* 41 (2): 163–170.

Marques MR, Santos IC, Carolino N, Belo CC, Renaville R, Cravador A. 2006. Effects of genetic polymorphisms at the growth hormone gene on milk yield in Serra da Estrela sheep. *Journal of Dairy Research.* 73(4): 394–405.

Ofir R and Gootwine E. 1997. Ovine growth hormone gene duplication–structural and evolutionary implications. *Mammalian Genome* 8: 770–772.

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. 1989. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphism. Proceedings of the National Academy of Sciences 86: 2766–2770.

Sambrook J, Fritsch EF and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Laboratory Press, Cold Spring Harbour, NY.

Theilmann JL, Skow LC, Baker JF, and Womack JE. 1989. Restriction fragment length polymorphisms for growth hormone, prolactin, osteonectin, crystalline, fibronectin and 21-steroid hydroxylase in cattle. *Animal. Gentics.* 20**:** 257.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, and Higgins DG. 1997. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*. 25: 4876–4882.

Wickramaratne SHG, Ulmek BR, Dixit SP, Kumar S and Vyas MK. 2010. Use of Growth Hormone Gene Polymorphism in Selecting *Osmanabadi* and *Sangamneri* Goats. *Tropical Agricultural Research* 21(4): 398 – 411.

Yao J, Aggre SE, Zadworn D, Hayes JF 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. G*enetics.* 144: 1809-1816.

Zhang C, Liu Y, Huang K, Zeng W, Xu D, Wen Q, and Yang L. 2011. The association of two single nucleotide polymorphisms (SNPs) in growth hormone (GH) gene with litter size and superovulation response in goat-breeds *Genetics and Molecular Biology* 34 (1): 49-55.