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Genetic variability of growth hormone gene and its association with growth traits in Sirohi breed of goat

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ABSTRACT

Genetic variations in the caprine growth hormone (c-GH. Gene) were investigated by single strand conformation polymorphism (SSCP) analysis of 8 amplified fragments covering almost the entire gene (approx 2.5 kb) in 188 Sirohi goats and was associated with body weights at birth, 3, 6 and 9 months of age. SSCP analysis revealed 4 to 8 unique banding patterns across 8 studied fragments of GH. The promoter and region having exon 3 showed higher level of polymorphism with 8 variants. The fragments consisting of exon 1, exon 4 and exon 5 revealed 6 variants. SSCP patterns in the promoter region had significantly influenced the birth weight. The SSCP variants in fragments consisting of exonic regions had also influenced the body weight at different ages in breed of goat. SSCP analysis has indicated the possibility of marker assisted selection for higher body weight at different ages in Sirohi breed of goat.

Key words: Association, Goat, Growth hormone gene, Growth traits, Polymorphism, Sirohi breed, Single strand conformation

Candidate genes are selected on the basis of known relationship between physiological or biochemical processes and are tested as putative quantitative trait loci (QTLs) (Yao *et al*. 1996). Current knowledge in production biology indicates that genetically superior animals differ from the inferior animals mainly in their regulation of nutrient utilization (Bass *et al.* 1991) and that growth hormone (GH) exerts a key control in nutrient use (Bauman 1992) and mammary development (Sejrsen *et al*. 1986). Growth hormone (GH) secreted by the pituitary gland is the major regulator of post natal growth and general metabolism (Bauman *et al.* 1988) thus affects growth rate (Breier *et al.* 1999, Li *et al.* 2004).

Thus, the GH gene is a promising candidate gene worth studying for its effects on growth-related traits in goats. Studies reported on the growth and carcass traits are relatively very few in caprine (Malveiro *et al.* 2001, Marques *et al.* 2003, Chitra and Aravindakshan 2004). The nucleotide sequence homologies between goat and bovine, porcine, human and rat were 98, 90, 74% and 72% respectively (Kioka *et al.* 1989). Genetic polymorphisms at the goat Growth Hormone gene have also been detected by single strand conformation polymorphism (SSCP) (Malveiro *et al.* 2001, Marques *et al.* 2003).

The objectives of the present study were first, to analyze the polymorphism of growth hormone gene to detect new mutations using single strand confirmation polymorphism (SSCP) and second, to investigate the association of SSCP variants with body weights at different ages in Sirohi breed of goat that could lead to the finding of genetic markers useful for improved selection of this breed for growth and other economic traits.

MATERIALS AND METHODS

Animals and DNA samples: Sirohi breed of goat is one of the important defined breeds of India (Acharya 1982) inhabited in the arid to semi-arid region of the country. It is commonly found in the districts of Sirohi and adjoining districts of, Rajasthan state and Gujarat state. It is popular among goat keepers due to its good performance for milk and meat production, high adaptability to adverse environmental conditions of the region and disease resistance (Verma *et al.* 2006).

Sirohi goats (188), the progeny of 18 sires maintained under All India Co-ordinated Research Project on goats at different farms under field conditions in Rajasthan State (India) were used in this study. The body weights of all the animals at different ages were collected. The blood samples

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(8–10 µl were collected from these animals by jugular vein puncture in vacuum tubes treated with 15% ethylene di-amine tetra acetic acid (EDTA) as an anticoagulant and stored at 4°C till processed. Genomic DNA was isolated from whole blood using phenol-chloroform method (Sambrook *et al*. 1989) with minor modifications.

DNA amplification with polymerase chain reaction (PCR): Based on the published nucleotide sequence information of goat growth hormone gene (Koika *et al*. 1989) pairs of oligonucleotide primer were synthesized to amplify eight fragments (designated GHP1, GHP2, GHP3, GHP4, GHP5, GHP6, GHP7 and GHP8). PCR primers were designed online for each gene under study using web based Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www_slow.cgi) based on published GenBank accession number D00476 covering a region of approx 2.5 kb. The primer sequence, location and size of amplified fragments are presented in Table 1. PCR reactions were performed using advanced primus 96 thermocycler using 200 mM each of dATP, dTTP, dGTP and dCTP; 50 mM Kcl, 10 mM Tris-Hcl (pH 9.0, 0.1% Triton X -100, 1.5 mM magnesium chloride; 0.75 unit of Taq DNA polymerase; 0.5

Table 1. Sequence and position of oligonucleotides and PCR analysis parameters

Fragment[a]	Primers	Primer sequence (5'-3')[b]	Length (bp)	$T_{Anneling}$ (C°)	Primers (pmol)/ DNA(ng)	Fragment location
5'U	GHP1 F	cccagggattaaacctgagtc	352	58	10/25	13–364
	GHP1 R	ctctgctgggccctttttat				
U-E1-I F	GHP2 F	gggggaaagggagagagaag	379	61	10/50	315–693
	GHP2 R	ccctagggagagaccaggag				
I 1-E2-I 2	GHP3 F	gatcaggcatccagctctct	396	58	15/50	635–1030
	GHP3 R	tcactgccttattcggaacc				
I 2-E3-I 3	GHP4 F	gatcaggcatccagctctct	334	61	5/50	1011–1344
	GHP4 R	tcactgccttattcggaacc				
I 3-E4-I 4	GHP5 F	agtggaggatgatggttggt	437	6I	15/50	1318–1754
	GHP5 R	ggtttcaaaacgggaagagg				
I 4-E5- D	GHP6 F	ctagcagcccagtcttgacc	388	61	10/50	1706–2093
	GHP6 R	ggggaggggtaacaacagat				
E5-D	GHP7 F	ctgcacaagacggagacgta	352	58	10/50	1993–2344
	GHP7 R	tcacagagaaggggatgtgc				
D	GHP8 F	gcacatccccttctctgtga	182	53	5/25	2325–2506
	GHP8 R	cttcccactcttggaggcta				

[a] U, Up stream region; E, Exon; I, Intron; F, forward; R, reverse; D, Down stream.
[b] Primers were designed according to published gGH gene sequence (Kioka *et al*. 1989).

Table 2. SSCP analysis parameters and the frequencies of different variants of Sirohi goat breed

Fragment (Name)	p.100 T	Run Temp (°C)	Number of patterns	Patterns and their frequencies (%)
5'UTR (GHP1)	12	10	8	AA (1.06), AB (5.31), AC (12.23), AD (17.02), AE (9.04), AF (12.23), AG (16.48), AH (22.87)
U-E1-I F (GHP2)	12	10	6	BA (14.89), BB (17.55), BC (18.08), BD18.61, BE (5.85), BF (3.19)
I 1-E2-I 2 (GHP3)	10	15	4	CA (45.21), CB (16.48), CC (12.76), CD (21.27)
I 2-E3-I 3 (GHP4)	12	10	8	DA (9.57), DB (13.29), DC (17.55), DD (9.04), DE (8.51), DF (23.93), DG (4.25), DH (6.38)
I 3-E4-I 4 (GHP5)	8	15	6	EA (43.61), EB (23.4), EC (2.65), ED (12.76), EE (4.78), EF (6.38)
I 4-E5- D (GHP6)	10	15	6	FA (16.48), FB (8.51), FC (19.68), FD (12.76), FE (23.4), FF (11.7)
E5-D (GHP7)	12	10	6	GA (12.23), GB (18.61), GC (29.25), GD (18.61).GE (11.17), GF (3.19)
3'UTR (GHP8)	12	19	4	HA (20.21), HB (19.68), HC (23.40), HD (27.65)

μM of each primers and 50–100 ng of genomic DNA in the final volume of 25µl). The amplification began with denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 56–61°C for 30s extension at 72°C for 30 s and final extension at 72°C for 5 min (Table 1). The amplified product of each fragment was analyzed by electrophoresis on 2% agarose gel (5V/cm) using ethidium bromide staining.

SSCP analysis: Five ml of each amplified product was added to 10 ml of stop solution (95% formamide, 10 mM NaOH, 0.05% xylene cylenol and 0.05% bromophenol blue, 20 mM EDTA) and denatured at 95°C for 5 min, snap cooled on crushed ice. 15–20 mL of each sample solution was loaded on 8–12% non-denaturizing polyacrylamide/TBE gel (Table 2). Gels were run at 25 W for 4–8 h depending upon the product length at 10–15°C in a 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.

Statistical analysis: The effect of different genetic variants on body weights at birth, 3, 6, and 9 months of age was studied with linear mixed effect model. The model included year (2003 to 2005) and season (summer and winter) of kidding, sex (male and female), cluster (1–4), kidding order (1–8), and their interactions, and haplotype (genetic variants) of the individual as fixed and animal genetic effect as random effects. Each trait was analyzed using derivative free restricted maximum likelihood method (DFREML computer program (Meyer 1989). with animal models and additive genetic relationship matrix. The linear model used to study the association under each fragment was as follows.

$$Y_{ikjlm} = m + S_i + K_j + G_k + P_i + e_{ijklm}$$

where, Y_{ikjlm} is the observation of the animal m on sex i with kidding order j and the sire conformation pattern of k and animal conformation pattern of l. m is the overall mean for each trait, S_i is the effect of sex (male, female), G_k is the genotype of the kth sire, P_j is the genotype of jth progeny. All effects in the model were treated as fixed except residual effect. The differences in average body weights at different ages across different genotypes were tested by paired‘t’ test.

RESULTS AND DISCUSSION

SSCP polymorphisms and statistical analysis: Eight GH fragments (GHP1 to GHP8), which covers almost the entire length of the goat GH gene, were amplified. SSCP variants and their frequencies in growth hormone gene in Sirohi breed of goat are presented in Table 2 and Fig. 1. The frequency of SSCP patterns varied within and across different studied fragments. The fragment GHP1 (5´ promoter region) and GHP4 (third intron and fourth exon) regions had highest (8) number of variants followed by GHP2, GHP5, GHP6 and GHP7 fragments which recorded 6 variants each and least

Table 3. Relationship of different variants of the GH gene with body weights (kg) at different ages

Fragment and variants		Association of different variants with			
		Birth weight	3 Month	6 Month	9 Month
GHP1	AA	2.25 ^{ab}	13^{a}	15^{a}	17.5^{a}
	AB	2.5 ^{ab}	13.2^{a}	16.45^{a}	19.45^{a}
	AC	2.421 ^{ab}	12.761^{a}	16.456^{a}	19.543^{a}
	AD	2.451 ^{ab}	12.871^{a}	15.903^{a}	19.387^{a}
	AE	2.227^{a}	11.9^{a}	16.133^{a}	19.133^{a}
	AF	2.372^{ab}	13^{a}	16.023^{a}	18.818^{a}
	AG	2.5^{ab}	13.1^{a}	16.167^{a}	19.3^{a}
	AH	2.567^{bc}	12.736^{a}	16.153^{a}	19.069^{a}
GHP2	BA	2.55 ^{abe}	12.769^{a}	15.5^{a}	18.558^{a}
	BB	2.596 ^{ab}	13.276^{a}	16.465^{a}	19.5^{ab}
	BC	2.509 ^{ace}	12.409^{a}	15.651^{a}	18.651^{ab}
	BD	2.481 ^{abcd}	13.045^{a}	16.394^{a}	19.288^{ab}
	BE	2.291 ^{abcdf}	12.833^{a}	16.667^{a}	20.042^{b}
	BF	2.166 ^{def}	13.083^{a}	16.833^{a}	20.167^{ab}
	BG	2.246 ^{f}	12.567^{a}	16.267^{a}	19.5^{ab}
GHP3	CA	2.443 ^{a}	12.737^{ab}	16.25^{a}	19.205^{a}
	CB	2.432 ^{a}	13.613^{a}	16.5^{a}	19.410^{a}
	CC	2.386 ^{a}	12.545^{ab}	16.045^{a}	18.841^{a}
	CD	2.518 ^{a}	12.5^{bc}	15.658^{a}	19.263^{a}
GHP4	DA	2.37 ^{a}	13.156^{ab}	15.906^{ab}	19.219^{acd}
	DB	2.442 ^{a}	12.687^{ac}	16.042^{ab}	18.771^{abc}
	DC	2.529 ^{a}	13.018^{a}	16.375^{ab}	19.375^{acd}
	DD	2.36 ^{a}	12.975^{a}	16.05^{ab}	19.225^{bcd}
	DE	2.483 ^{a}	12.583^{ac}	15.722^{ab}	18.5^{c}
	DF	2.514 ^{a}	13.093^{a}	16.616^{a}	19.895^{d}
	DG	2.333 ^{a}	11.278^{c}	14.778^{b}	18.056^{bc}
	DH	2.345 ^{a}	12.409^{ac}	16.136^{ab}	19.136^{bcd}
GHP5	EA	2.444 ^{a}	12.792^{a}	15.903^{a}	18.961^{a}
	EB	2.385 ^{a}	13.141^{a}	16.372^{a}	19.551^{ac}
	EC	2.4 ^{a}	11.6^{a}	14.8^{a}	17.7^{ab}
	ED	2.45 ^{a}	12.519^{a}	16.345^{a}	19.135^{ac}
	EE	2.589 ^{a}	12.444^{a}	16.222^{a}	19.333^{ac}
	EF	2.577 ^{a}	13.346^{a}	16.808^{a}	20.308^{c}
GHP6	FA	2.52 ^{a}	12.5833^{a}	15.617^{a}	18.783^{a}
	FB	2.294 ^{a}	12.971^{a}	16.029^{ab}	19.147^{a}
	FC	2.494 ^{a}	12.848^{a}	16.288^{ab}	19.364^{a}
	FD	2.4 ^{a}	12.82^{a}	16.1^{ab}	18.84^{a}
	FE	2.46 ^{a}	12.807^{a}	16.023^{ab}	19.273^{a}
	FF	2.45 ^{a}	13.025^{a}	17.05^{b}	19.975^{a}
GHP7	GA	2.532 ^{ac}	13.75^{ab}	16.932^{a}	20.114^{a}
	GB	2.47 ^{ac}	13.08^{b}	16.483^{ab}	19.7^{ab}
	GC	2.545 ^{a}	12.909^{ab}	16.164^{ab}	19.245^{b}
	GD	2.353 ^{ac}	12.029^{c}	15.691^{b}	18.809^{b}
	GE	2.286 ^{bc}	12.786^{bc}	15.786^{ab}	18.857^{b}
	GF	2.343 ^{ac}	12^{bc}	15.143^{ab}	17^{c}
GHP8	HA	2.484 ^{a}	13^{a}	16.432^{a}	19.378^{a}
	HB	2.453 ^{a}	12.794^{a}	15.956^{a}	19.103^{a}
	HC	2.467 ^{a}	12.5^{a}	16.044^{a}	19.189^{a}
	HD	2.413 ^{a}	12.981^{a}	16.123^{a}	19.179^{a}

*The figures having same superscripts don’t differ significantly ($p \leq 0.05$)

variants (4) were observed in GHP3 and GHP8 (3'UTR) region. The frequency of the patterns varied from 1.06 to 43.61% in all 8 fragments (Table 2).

The association analysis of variants of promoter region revealed that animals having AE variant had significantly lowest (2.227 kg) birth weight (P≤0.05) while AH variants indicated highest (2.567 kg) birth weight with an increase of 13.25% (Table 3). However, there was no difference found with the subsequent testing at 3 , 6 and 9 months of age. In fragment GHP2, BE variant recorded significantly highest (20.042 kg) body weight compared to BA variant (18.558 kg) at the age of 9 months. CB variant (13.613 kg) in fragment GHP3 showed 1.1 kg higher (P≤0.05) body weight than its counterpart (12.5 kg) having CD variant at three months of age.

Among the variants in fragment (Table 3) GHP4, DG variant indicated significantly lowest body weight at the age of 3 month and 6 month. It was 11.278 kg and 14.778 kg respectively. DF variant had highest body weight (19.895 kg) and compared to DE variant (18.5 kg) it is 1 kg weight extra at the age of 9 month. In fragment GHP5, EB variant recorded significantly (P≤0.05) highest body weight (20.308 kg) at the same age and it was 2.61 kg higher than the EC variant significantly (P≤0.05). Similarly, FF variant of the fragment GHP6 showed significantly higher body weight at the age of 6 months and it is 1.43 kg higher than its counterpart, FA variant. Among the variants of fragment GHP7, GC variant recorded higher birth weight (2.545 kg) and it had significantly higher birth weight than GE variant (2.286 kg). However, there were no differences in body weights at 3, 6 and 9 months of with respect to these two variants.

The SSCP analysis of the gGH gene revealed high level of polymorphism at promoter and across different exonic and intronic regions in this breed of goat. There is hardly any information available on association of gGH gene polymorphism with growth traits in goat except that of (Li *et al*. 2004) in Boer goats. They observed polymorphisms in the two fragments in 5´-region of gGH gene but no significant association with body weights was reported. However, they observed that goats with genotype DD had a higher body weight.

This investigation indicated that SSCP analysis is a valuable tool for the identification of genetic variants in the growth hormone gene in goats. Different SSCP patterns were identified in Sirohi breed of goat and variants in promoter and fragments consisting of exonic regions are significantly associated with body weights at different ages in this breed of goat. Sequencing of polymorphic fragments will allow the identification of the nature of the GH mutations detected by PCR-SSCP and provide information for the development of fast screening methods of a flock for further selection of possible relevant genetic variants. The study indicates the possibility of marker assisted selection for higher body weights at different ages in this breed of goat.

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