Association of the polymorphisms detected in β –lactoglobulin (β-LG) gene with milk production traits in Sirohi and Jamunapari breed of Indian goats

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ABSTRACT

The present investigation was carried out to explore the genetic polymorphisms in β –lactoglobulin (β-LG) gene and their possible association with milk production traits in two goat breeds of India. Total twelve SNPs were identified in caprine β –lactoglobulin (5'UTR, exon 1, exon 7 and 3'UTR) gene by screening 309 DNA samples of Jamunapari (140) and Sirohi (169) breed of goats using PCR - single strand conformation polymorphism (SSCP) and DNA sequencing. Out of twelve identified SNPs, four in 5'UTR (T1808C, C1866T, G2015T, C2084T and G2133A), one in intron 1(G3044A), one deletion at position 6591, two in exon 7 (G6751A, G6753C) and three in 3'UTR (T7011C, C7062A, G7147A) were detected. Association analysis revealed that the locus G2015T had significant effect on different milk production traits in Sirohi and Jamunapari goats. Locus T1808C and G6751A was found to be associated with milk yield in Jamunapari goats. The polymorphisms at the β –lactoglobulin associated with the milk production traits may be utilize as markers in selection of goats for high milk yield.

Keywords: β –lactoglobulin gene, SNP, Jamunapari, Sirohi, goat milk, SSCP
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INTRODUCTION

The domestic goat (Capra hircus) is an important livestock species in India. Due to small size and milk yield support for a small family (MacHugh and Bradley 2001) goats are extensively domesticated across the world particularly in the developing countries and serve as a vital resource of meat, milk, fiber (Zeder and Hesse 2000; MacHugh and Bradley 2001; Qureshi et al. 2014). The whey proteins of milk correspond to the protein fraction that remains in solution after precipitation of casein micelles and fat globules, and are constituted principally by β-lactoglobulin and α-lactalbumin. β –lactoglobulin (β-LG) is one of the major whey protein in ruminant milk. It is also found in the milk of other mammals, but absent from the milk of rodents, lagomorphs or humans (Hambraeus and Lonnerdal 2003). The biological functions of this protein are still not known. It could have a role in metabolism of phosphate in the mammary gland and the transport of retinol and fatty acids in the gut (Rachagani et al. 2006). Polymorphism of β-LG had been investigated in cattle and sheep, in which it has a remarkable effect on milk yield and composition (Tsiaras et al. 2005; Dario et al. 2008). Similarly, in goat, several alleles had been discovered at both DNA and protein levels (Pena et al. 2000; Yahyaoui et al. 2000; Graziano et al. 2003; Ballester, 2005). However, in Indian goats the effect of polymorphism of β-LG on milk yield and other milk quality traits is not yet clear. Hence, the aim of this study was to detect single nucleotide polymorphisms (SNPs) in the β-LG gene and to determine their influence on milk production traits in Sirohi and Jamunapari breeds of Indian goat.

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 -20oC 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.

Primer synthesis and PCR amplification

Primers were designed based on published goat β-LG sequence (GenBank accession number Z33881) by PRIMER3 software available online (http://frodo.wi.mit.edu/cgibin/primer3/primer3_www_slow.cgi). 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 Bio System). 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 β-LG 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 β-LG 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

Different fragments of β –lactoglobulin (β-LG) gene were amplified. Based on distinct band pattern in SSCP, the individuals representing unique variants selected for sequence analysis revealed T/C, C/T, G/T, C/T and G/A substitutions at 1808, 1866, 2015, 2084 and 2133 positions respectively falling under the promoter- 5' UTR region (Table 1) in both the of the breeds under investigation.

In the similar study showed polymorphism in the 5' flanking region of the β-LG gene including 6 single nucleotide substitutions, a single nucleotide deletion, and a 7 bp duplication in cattle by Branschweig (2007). The exon 1 region was observed fully conserved region in both the breeds. One single nucleotide polymorphic site G/A at position 3044, located in the intron 1 region of β-LG gene. One deletion (- A) was observed at position 6591 in intron 6 in both of the breeds. Two SNPs G/A and G/C were observed in exon 7, at positions 6751 and 6753 respectively in both the breeds. Sequence analysis of 3' UTR revealed T/C, C/A and G/A

Table 1. Age and sex wise different biometric traits (cm) in Indigenous cattle of Manipur

Fragment/Region	Nt. Position	Sequence change	Sequence	Reference sequence	Breed change	Amino Acid
Promoter-5' UTR	1808	T/C	CTCCAGG	CTCTAGG	S*/J*	-
(1618-2068)	1866	C/T	CCCTGGA	CCCCGGA	S/J	
	2015	G/T	CTCTTAG	CTCGTAG	S/J	
	2084	C/T	GCCTGGC	GCCCGGC	S/J	
	2133	G/A	CCCACTG	CCCGCTG	S/J	
Intron 1	3044	G/A	TCAAAGA	TCAGAGA	S/J	-
Intron 6	6591	A/-	CTG-ACC	CTGAACC	S/J	deletion
Exon 7	6751	G/A	ACCACGG	ACCGCGG	S/J	No change
	6753	G/C	CGCCGTC	CGCGGTC	S/J	No change
3'UTR	7011	T/C	TCTCTGA	TCTTTGA	S/J	-
	7062	C/A	TAAACTT	TAACCTT	S/J	-
	7147	G/A	TATAAAA	TATGAAA	S/J	-

S*- Sirohi, J*- Jamunapari

substitution at 7011, 7062 and 7147 nucleotide position respectively. Ballester et al. (2005) reported 15 β-lactoglobulin polymorphisms in different goat breeds from Spain, France, Italy-Switzerland, Senegal and Asia. Nine, of the fifteen polymorphisms were located in the promoter region and six in the exons of the gene. All polymorphisms are single nucleotide substitutions with the exception of one deletion/insertion in the promoter region.

Association analysis

The locus T1808C of 5'UTR found to be significantly associated with fat percentage in Jamunapari goats. The animals with genotype CC were superior to the animals with genotype TT by 1.48% at fat percentage (Table 2). The contribution of the locus to total phenotypic variability for fat percentage was observed 1.43%. The genotypes at locus T2015G of β-lactoglobulin gene significantly influenced the protein, lactose and SNF (%) in Jamunapari goat (Table 2) but in Sirohi the locus T2015G influences only lactose and SNF (Table 3). The contribution of the position to total phenotypic variability varied from 8.24 (protein %) to 3.61% (lactose %) (Table 2). The animals with genotype 'TT' were superior to the animals with genotype 'GG and GT' by 3.69 and 9.09% for protein (%), by 5.00 and 8.65% for lactose (%) and 3.86 and 9.18 (%), for SNF (%), respectively in *Jamunapari* goats. One variant was found by Chen et al. (2005) in the β-LG gene's 5' flanking region (710 bp) in Xinong Saanen dairy goats. The study showed that milk yield of individuals with genotype AA was higher than that with genotype AB in second and third lactation milk yield and average milk($P<0.05$). The results implied that allele A of β-lactoglobulin genes in 5' flanking region is probably related to high milk protein yield. Genetic polymorphisms in the 5' flanking region (promoter region) was also explored by Yahyaoui et al. (2003) who analyzed goats of Murciano-Granadina, Canaria Payoya, Malaguena and Saanen breeds, but no effect of this variant either on transcription level or on the protein were found yet, although it must be noted that the most frequent variant is the same for all brands. However, in the Girgentana goat, an individual variability in β-lactoglobulin content has been observed (Chianese et al. 2000), and subsequently the Girgentana gene promoter region was characterised and a new polymorphism not related to the β-lactoglobulin content was detected (Graziano et al., 2003).

Locus A6751G of exon 7 of β-lactoglobulin gene influenced 90 Days milk yield significantly in

Table 2. Least-square analysis of different milk production traits of various genotypes of β-lactoglobulin gene in Jamunapari goats

Position	Fragment	Genotypes (N)	Fat (%)	Protein (%)	Lactose (%)	SNF (%)	90DMY (l)	140DMY (l)	TMY (l)	LL (days)
C1808T	5'UTR		S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
		CC (67)	3.42±0.28	3.40±0.05	5.01±0.08	9.25±0.16	78.13±3.96	120.74±5.73	146.92±7.36	198.09±3.62
		TT (73)	3.12±0.27	3.47±0.05	5.14±0.08	9.45±0.15	87.91±3.77	125.45±5.46	154.49±7.03	203.14±3.45
	P value		0.024							
	R^2 Value		1.43							
T2015G										
			N.S	S	S	S	N.S	N.S	N.S	N.S
		TT (48)	3.60±0.26	3.52±0.05a	5.20±0.08a	9.58±0.14a	85.04±3.80	127.06±5.40	155.39±6.98	199.78±3.43
		GG (52)	3.29±0.32	3.39±0.06ab	4.94±0.09ab	9.21±0.18ab	79.99±4.66	118.71±6.62	145.62±8.55	199.78±4.20
		GT (40)	2.95±0.60	3.20±0.11b	4.75±0.18b	8.70±0.33b	85.18±8.52	118.94±12.10	145.92±15.63	208.52±7.68
	P value			0.031	0.041	0.036				
	R^2 Value			8.24	3.61	7.93				
A6751G	Exon 7									
			N.S	N.S	N.S	N.S	S	N.S	N.S	N.S
		AA (29)	3.03±0.32	3.38±0.06	5.00±0.10	9.20±0.18	89.13±4.51ab	129.63±6.57	160.14±8.44	204.74±4.17
		GG (67)	3.51±0.28	3.44±0.05	5.09±0.08	9.37±0.16	75.07±3.96a	117.00±5.77	141.66±7.41	196.91±3.66
		GA (44)	3.98±0.45	3.51±0.09	5.22±0.14	9.63±0.26	90.17±6.39b	126.52±9.30	156.35±11.95	202.65±5.91
	P value						0.018			
	R^2 Value						6.90			

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)-liters

Table 3. Least-square analysis of different milk production traits of various genotypes of β-lactoglobulin gene in Sirohi goats.

SNP	Genotype (N)	Fat	Protein	Lactose	SNF	LL	TMY
T2015G		N.S.	N.S.	S	S	N.S.	N.S.
	TG (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
	GG (34)	4.23±1.10	3.52±0.10	5.15±0.15b	9.48±0.27b	153.44±1.11	96.31±3.23
P value				0.043	0.038		
R 2 Value				3.18	4.26		

Values with different superscripts in the same column are significantly different at P≤ 0.05, N.S.= Non-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.

Jamunapari goats. The contribution of this locus to total phenotypic variability was 6.9 % for 90 days milk yield (Table 2). The animals with genotype GA were superior to the genotype GG and AA by 16.74 and 1.15 % for 90 days milk yield, respectively in Jamunapari goats. . The other polymorphic position contain a 10 bp long insertion at position +4641, that can be detected by capillary electrophoresis of the PCR product amplified with a fluorescent primer (Kumar et al. 2006) by SDS-page analysis observed two alleles having an effect on 90-day milk production. The results indicate that selection had been effective in increasing proportion of favorable alleles for higher growth and milk production traits in both breeds in breeding programs.

CONCLUSION

The polymorphisms that found to influence on milk production traits in β-lactoglobulin gene in two breeds of Indian goat may be utilize for marker assisted selection of goat for superior milk quality and milk yield. As the in β-lactoglobulin gene is exceptionally versatile responsible for promoting several functions including regulation of postnatal somatic growth, stimulating anabolic processes such as cell division, skeletal growth and protein synthesis, the identified SNPs may need to be associated with other economic traits (production, reproduction, health, management and physical appearance traits). As the findings on association studies were based on the limited number of sample data, reconfirmation of findings are suggested with adequately high number of samples and précised set of data.

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