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β-Lactoglobulin gene polymorphism in Indian sheep breeds of different agro-climatic regions

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β-lactoglobulin (β-LG) protein coded by (β-LG) gene is one of the major whey protein present in ruminants milk representing about 50% of the total protein. β-LG gene is located on chromosome 3 with 4.9 Kb long transcription units comprising 7 exons and 6 introns. As β-LG is able to bind a variety of hydrophobic molecules including retinol and small fatty acids, its role in transport of vitamin A was also proposed. Milk protein polymorphism can serve as a marker and have been considered as a potential tool for selection of dairy ruminants. It is one of the several positional candidate genes which may affect milk production traits in sheep. The polymorphism is due to single nucleotide substitution in exonII (T–C), expressed as a change of the 20th amino acid (Tyr to His). This substitution disrupts *Rsa*I site which can be detected by PCR-RFLP. Allelic variants A and B differ by a Tyr/His substitution, where β-LG A has Tyr and *β-LG* B has His (Kolde and Braunitzer 1983). The rare variant β-LG C is a subtype of ovine β-LG A with a single exchange of Arg-Glu (Erhardt 1989). Several studies has been carried out to study polymorphism in ovine β-Lactoglobulin gene (Cubric-Curik *et al*. 2002), Barillet *et al*. (2005), Kucinskiene *et al.* (2005), Cengiz *et al*. (2006), Dario *et al*. (2005), Dario *et al*. (2008), Baranyi *et al*. (1993), Arora *et al*. (2010).

Ovine milk has a unique whey protein composition as compared to that of bovine whey and it is especially rich in proteins (Moatsou *et al.* 2005). The uniqueness of sheep milk and its cheese production characteristics, has spurred considerable research into genetic structure of milk related genes in sheep populations. Milk production of an ewe has an important effect on the pre-weaning growth and survival of lambs especially in case of twins and triplets. Improvement in milk producing ability of ewes and lamb survival will result in significant economic returns to most of sheep production systems. Reported relationship between genetic variants of β-lactoglobulin gene and milk yield, composition and cheese-making ability have raised interest for the polymorphism identification of β-LG in dairy sheep populations. AA and AB genotypes are associated with casein content and curd yield whereas BB genotype is associated with a significantly higher milk yield. In modern breeding programmes, polymorphisms of the milk protein genes can be used as marker systems.

Considering the importance of this gene, it is important to determine the superior genotypes in native Indian sheep breeds and develop breeding programmes and selection strategies. So, this study aims to identify allelic variants of β-LG gene in ten native Indian sheep breeds of different agro-climatic regions of India by PCR-RFLP method.

Sample collection: Blood samples were randomly collected from 432 animals belonging to the 10 native Indian sheep breeds [Patanwadi (44), Kendrapada (45), Dumba (43), Garole (42), Malpura (44), Madgyal (46), Nali (42), Deccani (41), Chokla (43), Sonadi (42)] from their breeding tracts varying in agro-climatic regions.

Genomic DNA Extraction: Approximately 5ml of blood samples were collected from all animals aseptically from jugular vein in ACD (citric acid, sodium citrate, D-glucose) solution in 15ml centrifuge tubes. DNA was extracted from white blood cells using standard phenol-chloroform extraction method (Sambrook and Russell 2001) with minor modification. DNA samples were dissolved in 0.1X TE buffer (pH 8.0).

PCR Amplification and genotyping: PCR Amplification of exon II fragment of ovine β-LG gene was done using forward: (5'CAACTCAAGGTCCCTCTCCA3') and reverse (5'CTTCAGCTCCTCCACGTACA 3') primers as reported by Arora *et al.* (2010). The PCR reaction was carried out in 25µl of total volume, containing 10X PCR buffer (50mMl/l KCl, 10 mM/l TRIS–HCl pH 8.0, 0.1% Triton X-100), 2mM $MgCl_2$, 0.2mM of each dNTP, 5 pM/lm of each primer, 100–150 ng ovine genomic DNA and 1U Taq DNA polymerase. Amplification was performed in Mastercycler Gradient programmed for initial denaturation

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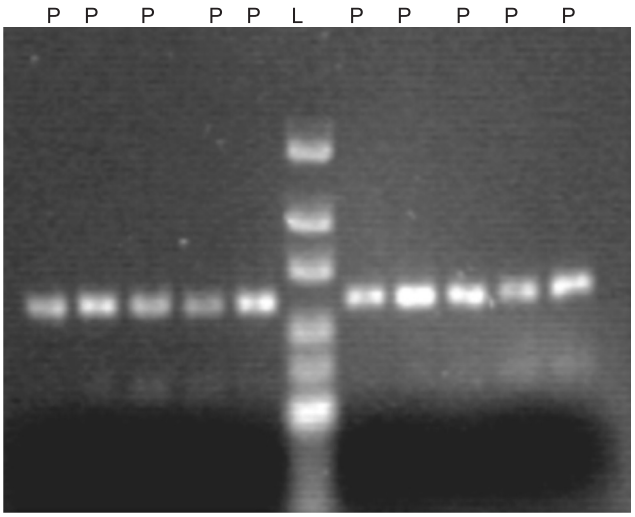

Fig. 1. PCR amplification of β-LG gene resolved on 3% agarose gel : Lanes L : 100bp DNA ladder and P-PCR product (120bp).

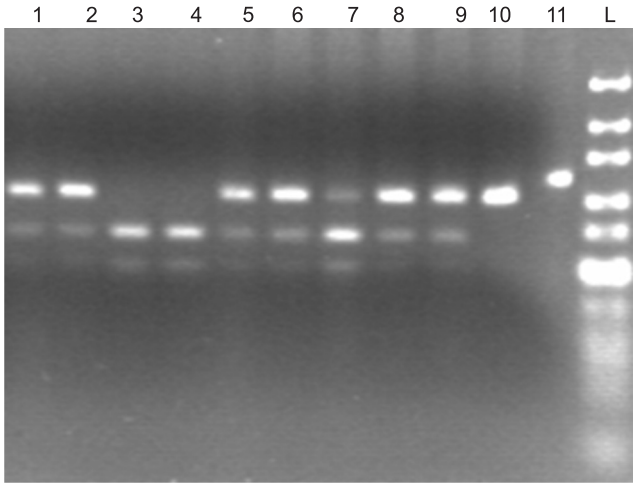

Fig 2. PCR-RFLP pattern of β-LG gene as resolved on 4 % agarose gel: Lanes 1, 2, 5, 6, 7, 8 and 9 represents AB genotype (103, 66, 37 and 17 bp), lanes 3 and 4 represents AA genotype (66, 37 and 17 bp) and Lanes 10 and 11 represents BB genotype (103 and 17bp), lane L: 50 bp DNA ladder.

at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 15 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec, and final extension at 72°C for 10 min. PCR products were checked for amplification by electrophoresis on 3% agarose gel, in parallel with 100 bp DNA marker. Genotyping was done using PCR-RFLP analysis as described by Feligini *et al.* (1998). Digestion of a 120-bp PCR fragment of *β-LG* exon II was done with restriction endonuclease *Rsa*I. All the PCR products were digested separately with 5U of RsaI enzyme in a 15µl total reaction volume at 37°C overnight. The digested products after digestion were resolved by electrophoresis on 4% agarose gel in parallel with a 50 bp DNA marker. The genotype patterns were visualized under UV light after staining with ethidium bromide. Allele discrimination was based on size differentiation (bp) of respective alleles in β-LG gene.

Statistical analysis: Allele and genotype frequencies were estimated for the genetic variants of β-LG gene by direct counting method. The actual distribution of genotypes in the analysed sheep populations with theoretical distribution was verified by chi-square test using Popgene ver.1.3. Nei's (1972) genetic identity and genetic distance was calculated using Popgene ver.1.3. Dendrogram based on Nei's (1972) genetic distances was drawn based on unweighed pair group method using arithmetic averages (UPGMA) for the β-LG alleles.

Polymorphism was found in all the sheep breeds: Two genetic variants (A and B) and three genotypes (AA, AB, and BB) were identified as reported earlier in other native Indian sheep breeds by Arora *et al.* (2010). Three different genotypes, AA (66, 37 and 17 bp), AB (103, 66, 37 and 17 bp) and BB (103 and 17 bp) were detected (Fig 2). Allele frequencies and genotype frequencies for β-LG gene in native Indian sheep breeds are given in Table 1.

Overall frequency of Allele A and B in studied breeds was (0.59) and (0.41) respectively. The allele A predominated in the majority of the studied breeds except Patanwadi and Kendrapada. Previously Recio *et al*. (1997), Dario *et al*. (2005), Mohammadi *et al*. (2006) also reported higher frequency of A allele in different sheep breeds.

Table 1. Distribution of β-LG genotype and allele frequency in Indian sheep breeds

Breed (N)	Allele frequency		Genotype frequency			Chi-square	P value
	A	B	AA	AB	BB	χ^2	
Patanwadi (44)	0.41	0.59	0.29	0.24	0.47	11.55	0.0006
Kendrapada (45)	0.48	0.52	0.37	0.22	0.41	13.43	0.0002
Dumba (43)	0.95	0.05	0.93	0.05	0.02	13.15	0.0002
Garole (42)	0.62	0.38	0.40	0.43	0.17	0.450	0.5020
Malpura (44)	0.51	0.49	0.39	0.25	0.36	11.50	0.0007
Madgyal (46)	0.50	0.50	0.20	0.60	0.20	1.959	0.1600
Nali (42)	0.63	0.37	0.41	0.45	0.14	0.069	0.7910
Deccani (41)	0.50	0.50	0.27	0.46	0.27	0.299	0.5839
Chokla (43)	0.59	0.41	0.49	0.21	0.30	14.40	0.0001
Sonadi (42)	0.71	0.29	0.48	0.48	0.04	1.01	0.3130
Mean	0.59	0.41	0.42	0.34	0.24		

Barillet *et al*. (2005) also reported similar frequency of A allele as 0.58, 0.64 & 0.68 in Merino, French lacaune and Spanish sequerena breeds respectively. Our findings were in contrast to other native Indian breeds as reported by Arora *et al*. (2010) where B allele predominated with average frequency of 0.63 for Allele B and 0.37 for A allele. However, only four breeds namely Chokla, Sonadi, Deccani and Garole were common to both the studies out of which Chokla and Deccani has higher frequency of A allele in both the studies. The frequency of Allele A ranged from 0.41 to 0.95 and Allele B ranged from 0.05 to 0.59. The frequency of A allele was lower than in other Turkish, Egyptian and Lacunae sheep breeds (Cengiz *et al.* 2006, Dario *et al.* 2008, and Othman *et al.* 2012).

The frequencies of 3 genotypes ranged (0.20–0.93), (0.05–0.60) and (0.04–0.47) for AA, AB and BB respectively. The most common genotype at the β-LG locus was AB which predominated in Madgyal, Sonadi, Nali, Garole and Deccani breeds. Higher AB genotype frequency was also observed by Cubric-curik *et al.* (2002) in Croatian Pag sheep, Piwezynski *et al.* (2002) in Polish merino and in crosses derived from Finn, Romanov and Boorola rams and Mohammadi *et al*. (2006) in various Iranian and Russian sheep breeds. The second most abundant genotype was AA whereas BB genotype was least prevalent. AA genotype was higher in Dumba, Malpura and Chokla breeds, BB genotype was found to be higher in Patanwadi and Kendrapada. Genotype frequency pattern was similar to most of the other findings and in contrast to other Indian sheep breeds as studied by Arora *et al.* (2010) where AA genotype was least prevalent. All of the breeds were in agreement with Hardy-Weinberg Equilibrium except Patanwadi, Dumba, Malpura, Kendrapada and Chokla breed. The deviation from Hardy-Weinberg Equilibrium in these breeds may due non random mating and selection being practised. Jyotsana *et al*. (2010) reported crossing of Dumba rams with Marwari and Patanwadi breeds because of its superior production trait (ability to attain higher body weight and better milk production of ewes). Genetic relationship between breeds based on the information provided from polymorphism of β-LG are represented in a dendrogram (Fig-3). Deccani sheep represented a distinct

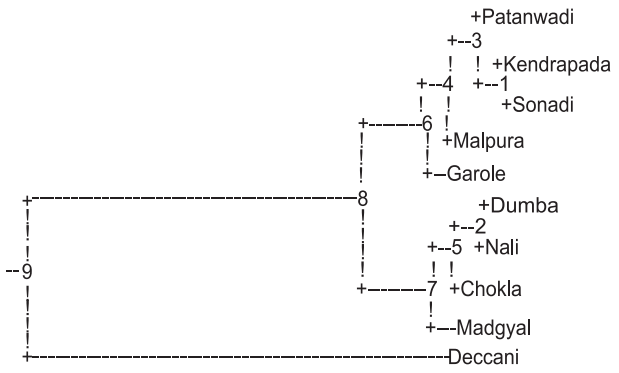

Fig. 3. Genetic distance clustered by the UPGMA for the β-LG alleles in Indian breeds.

population among other sheep breeds. Among indigenous breeds Patanwadi, Kendrapada, Garole, Malpura and Sonadi formed distinct cluster from other breeds which clearly depicts their utility as a dual purpose breeds and their milk producing ability.

SUMMARY

Two genetic variants (A and B) and three genotypes (AB, BB and AA) were found in studied sheep breeds. The average frequency of A and B allele was (0.59) and (0.41) respectively. The A allele was more frequent among the studied breeds except Patanwadi and Kendrapada breeds. There was no clear cut predominance of any of the genotype. The Patanwadi, Malpura, Dumba, Kendrapada and Chokla populations were not in Hardy-Weinberg equilibrium. These results suggested the presence of β-LG gene polymorphism and predominance of β-LG A type in majority of the studied breeds. From the above findings it may be concluded that the native sheep breeds depicts variation in β-LG exon II locus. Further, genotype and allele distribution pattern in different breeds may be due to different characteristic of milk of these breeds. The contrasting pattern of variation observed in present study compared to previous study in native sheep breeds also highlight the need for further studies using large number of animals from different geographic regions. The relationship between β-LG genetic variants and traits related to milk and cheese production characteristics in rest of Indian sheep breeds need to be explored.

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