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SNP analysis of growth differentiation factor 8 *(GDF8)* gene in Indian sheep

R ARORA[1], D K YADAV[2] and H S YADAV[3]

National Bureau of Animal Genetic Resources, Karnal, Haryana 132001 India

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The indigenous sheep, valued for wool and mutton, have a range of diversity, excellent adaptive traits, hardiness and disease resistance, which make them economical in one production system. Mutton and lamb form a relatively small segment of the mutton industry where demand is outstripping supply. The production levels are almost constant at 950,000 MT as the market for mutton lies unexplored. Muzaffarnagri, Sonadi, Garole, Deccani and Mandya, economically important breeds, represent a vast gene pool with untapped genetic potential for commercial purposes and for maintenance of genetic diversity in this species. The identification of unique genes may also lead to development of potential markers that can be used in selective breeding programmes. Breeds having economic value will be automatically conserved by the farmers. The most important challenges in the near future are the identification of useful variation (real or potential) in germplasm and its use in guiding conservation decisions. Almost 50% of the indigenous breeds are declining and some are even threatened for extinction (Bhatia and Arora 2005). For domestic species an indication of the extent of variation, especially at loci of economic importance, may provide information on functional differences between breeds which is relevant for selective breeding programmes to establish new breeding stocks for maintenance of genetic diversity (Parsons *et al*. 1996). Although Indian sheep have been widely studied for neutral variations (Arora *et al.* 2011) few reports are available on genetic variation at functional loci (Arora *et al.* 2008, 2010).

The growth differentiation factor 8 *(GDF8)* or *Myostatin* gene located on ovine chromosome 2, is a candidate gene influencing mutton production. It is a member of transforming growth factor beta super family and a negative regulator of muscle growth. In sheep, single nucleotide polymorphisms (SNPs) identified in the *GDF8* gene were found associated with muscle depth (Clop *et al.* 2006), increased forequarter weight, mutton tenderness (Kijas *et al.* 2007), lean meat yield (Johnson *et al.* 2009) and loin yield/ proportion (Hickford *et al.* 2010). Several other nucleotide variations were reported in this gene (Zhou *et al.* 2008) revealing the diversity available within this gene. Data pertaining to SNPs/polymorphism at *GDF8* gene in Indian sheep is completely lacking. The main aim of the present study was to identify sequence variants in the promoter, exon 1, intron1, exon 2 and intron 2 regions of ovine *GDF8* gene in Indian sheep.

For nucleotide diversity analysis of the *GDF8* gene a panel of 45 DNA samples including 9 sheep breeds (Muzaffarnagri, Malpura, Magra, Nali, Chokla from Northwestern arid and semi arid region, Deccani, Madgyal from Southern peninsular region and Ganjam, Garole sheep from eastern region of India) was utilized. Genomic DNA was isolated from blood samples and purified using the standard phenol chloroform extraction protocol (Sambrook *et al.* 1989). The genomic DNA samples were subjected to PCR amplification followed by direct sequencing. The promoter, exon 1, 2 and intron 1, 2 regions were amplified using reported primers (Kijas *et al.* 2007). For the PCR amplification, about 100ng of genomic DNA was taken as template in a 25µl PCR reaction containing 10pmol of each primer, 200µM dNTPs, 1U Taq polymerase and 1.5 mM $MgCl_2$, PCR cycling consisted of 30 cycles of 95ºC, 61–66 ºC annealing temperature (for different loci), 72 ºC for 1 min each, followed by a final extension step of 72ºC for 7 min. The amplicons were visualized by ethidium

Table 1. Genotype and allele frequencies of SNPs identified in *GDF8* gene in Indian sheep

Region	SNP	Genotype frequency	Allele frequency
Promoter	-41C>A	AA - 0.154	A-0.411
		CC - 0.333	C-0.589
		AC - 0.513	
- do -	-16 C>T	CC - 0.000	C-0.064
		TT - 0.872	T-0.936
		CT -0.128	
Intron 1	+391G>T	GG - 0.585	G-0.756
		TT - 0.073	T-0.244
		GT- 0.342	

Present address: [1,2]Senior Scientist (rejagati@yahoo.co.in, dkyadav66@gmail.com),[3]SRF (hsyadav@gmail.com).

Table 2. Comparison of SNPs identified in the *GDF8* gene in Indian sheep with exotic sheep

SNP	Region	Exotic Sheep	Indian Sheep	Association	Ref.
-41C>A	Promoter	Yes	Yes	Fore quarter wt, mutton tenderness	Kijas *et al.* (2007)
-39T>C	Promoter	Yes	Singleton	-	Kijas *et al.* (2007)
–16 C>T	Promoter	No	Yes	-	This study
+391G>T	Intron 1	Yes	Yes	Leg, loin, total yield	Hickford *et al.* (2010)

bromide staining after electrophoresis in 1.5% agarose gel. The amplicons were purified by ExoSap and sequenced on genetic analyzer. Multiple sequence alignments and comparisons were carried out using software programmes (Tamura *et al.* 2007). Genotypes were determined by direct counting. The allelic frequencies were determined by counting the number of alleles and dividing by the total number of alleles.

The analysis of 1004bp of the partial coding and non coding region of the *GDF8* gene, among 45 DNA samples sequenced from a total of 9 different sheep breeds allowed the identification of several nucleotide sequence variants. Sequence alignments revealed that the consensus sequence in Indian sheep shared 99% homology with the reference sequence (DQ530260, Clop *et al.* 2006). Mutations/variations were scanned within the exon 1, 2 and intron 1, 2 regions of the *GDF8* gene. Three SNPs, 2 in promoter (g-41 C>A; g-16 C>T) and one in intron 1 (g.+391G>T) region, i.e. 2 transversions and one transition were observed (Table 1). Seven singletons and 2 indels were also detected. All the identified SNPs were present in the heterozygous state. No SNPs were detected in the region of the exon 1, exon 2 and partial intron 2, although some singletons and indels were observed.

The frequency of the C allele (g.-41C), associated with increased forequarter weight and mutton tenderness (Kijas *et al.* 2007) across all the 9 Indian sheep breeds investigated, was 0.589, whereas frequency of T allele (g+391T) associated with increased leg, loin and total yield (Hickford *et al.* 2010) was only 0.244 in Indian sheep breeds (Table 1). Frequency of the favourable allele C at SNP g- 41C>A was higher than that of A. Similarly frequency of allele G for SNP +391 G>T was higher than that of T. The results revealed 2 previously described SNPs which were known to be associated with mutton and carcass quality traits, as well as 1 new SNP, which was specific to Indian sheep breeds investigated in this study (Table 2).

The SNP g–16 C>T, discovered in this study in the promoter region, has not been previously reported. All the individuals genotyped at this SNP were either heterozygous (C/T) or homozygous TT, no homozygous CC individuals were observed. However, the frequency of the heterozygotes (C/T) was only 0.128.

The allele frequency profile of -41C>A across exotic and Indian sheep revealed the existence of the C allele in more than 50% of the Indian sheep breeds investigated. The presence of the favourable allele, associated with increased forequarter weight and mutton tenderness suggested its dispersal in Indian sheep too. The results obtained reflected that there is substantial prospect for improving genetic gain in Indian sheep through selective breeding.

SUMMARY

This study demonstrates genetic polymorphism in *GDF8* gene in limited samples of 9 Indian sheep breeds. Two SNPs were identified in the Indian sheep which are known to be associated with mutton quality and carcass traits in exotic sheep. The need of the hour is to characterize our own breeds and utilize their genetic potential for improvement of mutton quality. These indigenous genetic resources are underutilized in conventional breeding programmes due to failure to identify animals carrying the most advantageous traits. Identification of genes controlling traits of economic importance and subsequent use of this information in selection and breeding programmes may offer substantial gains in productivity. These initial results showing genetic variability and presence of favourable SNPs in the *GDF8* gene may lead to exhaustive analysis of genetic polymorphism in *GDF8* gene in Indian sheep breeds.

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