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Original Article

Polymorphism studies of Prolactin Receptor (PRLR) gene in Indigenous Grey Cattle breeds of India

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Abstract

Alterations in candidate genes by way of substitutions, insertions, or deletions at restriction enzyme cleavage sites may produce changes in the resulting length of DNA fragments called restriction fragment length polymorphisms, which may serve as genetic markers if they are linked to quantitative trait loci. Recently, single nucleotide polymorphisms (SNPs) have gained high popularity as markers for genetic studies due to their high accuracy and reproducibility. The present study was undertaken to detect polymorphism in Exon 10 region of Prolactin Receptor (PRLR) gene. We have detected two novel SNPs in Exon 10 region of Prolactin Receptor (PRLR) gene in 10 indigenous grey cattle breeds from different agro-climatic regions of India. The PCR product using a set of primer pairs were digested with *Nae*I and *Sml*I restriction enzymes resulting in fragments of 232 bp and 168 bp from exon 10 region of PRLR gene. Out of 455 animals screened, 75.8% animals were homozygous AA, 3.1% of animal were homozygous CC and 21.1% animals were heterozygous (AC). The frequency of C allele was significantly lower than the A allele in all the cattle breeds studied. The mean gene frequency of C allele was estimated to be 0.136 with Kangayam showing the maximum frequency (0.213), and Punganur the minimum frequency (0.042). For the second set of primer out of 454 animals were screened, GT genotype was most frequent with genotypic frequency of 43.2%, followed by homozygous GG genotype with a frequency of 43.0% and TT with 13.8% frequency. The frequency distribution of G allele across the ten breeds ranged from 0.446 (Nagori) to 0.771 (Hill Cattle) with a mean value of 0.645, whereas that of T allele ranged from 0.229 (Hill Cattle) to 0.554 (Nagori) with a mean value of 0.355.

Keywords: Prolactin receptor, Polymorphism, Milk, Traits.

Introduction

In dairy cattle certain genes are proposed as potential candidates associated with dairy performance traits in marker-assisted selection (MAS). Among different candidates, Prolactin receptor gene (PRLR) seems to be promising because of its crucial role in transmitting signal from lactogenic hormones to milk protein gene promoters. Prolactin Receptor (PRLR) is the specific receptor for prolactin, which is an anterior pituitary peptide hormone involved in different physiological activities and is essential for reproductive success [5, 12]. It binds prolactin and contributes to activation of JAK2 kinases and subsequent phosphorylation of STAT5 transcription factors which bind to recognition sequences located in promoters of milk protein genes [5]. Therefore, PRLR is suggested as candidate gene associated with milk protein yield and content in dairy cattle.

Several polymorphic sites have been detected within PRLR gene and statistically significant associations between PRLR variants and milk production traits have been described in dairy cattle [9, 11, 15]. To our knowledge, the studies confirmed statistically significant associations between PRLR variants and milk production, reproduction performance in livestock [1, 2, 3, 4, 9, 10, 11, 15]. Recently, single nucleotide polymorphisms (SNPs) have gained high popularity in genetic studies due to their abundance, high accuracy and reproducibility. Therefore studying candidate gene polymorphisms in exons or other important regions, such as promoters utilising SNPs provides an appropriate approach.

The objective of this study was to identify and characterize polymorphisms in exon 10 of PRLR gene in ten native indigenous grey cattle breeds. The present study argues for the involvement of some additional alleles or genes influencing milk quality and production traits. There is need to investigate the Indian cattle with respect to various SNPs having associations with phenotypes of economically traits which can be used for their improvement.

Materials and Methods

Data Collection

A total of 460 blood were collected randomly from genetically unrelated animals of Hariana, Nagori, Tharparkar, Mewati, Binjharpuri, Ghumusari, Kangayam, Hill Cattle, Punganur and Kankrej cattle breeds from their respective native breeding tracts. Whole blood (7-8 ml) was collected from jugular vein in EDTA coated vacutainer tubes. The blood samples were transported from the field to laboratory at 0^{o}-5^{o}C and genomic DNA was extracted from the blood tissue according to the standard phenol and chloroform method [8].

Designing primers for the region of interest and SNP detection using PCR-RFLP

Two pairs of oligonucleotide primers were synthesized to amplify two different fragments of 232 bp and 168 bp in length, based on available sequences of the bovine PRLR gene exon 10 (Gene Bank accession number: FJ901285, SNP location 486bp and 828bp) using PRIMER-3 software (http://frodo.wi.mit.edu/). The primer sequences used for the 232-bp fragment containing a polymorphic *Nae*I restriction site at the 125 position were Forward Primer CCTATTTTCTGGCCAATGGA; Reverse Primer TCTGACTCCCTCTGCTTGGT, and for the 168-bp fragment containing a polymorphic *Sml*I restriction site at the 123 position were Forward Primer AGATGGAGTGCTGGCTCTGT; Reverse Primer GCCTTCTTGGCTGGTTCTTC.

The PCR was performed in a volume of 25µl using 1.5 mM $MgCl_2$, 200 µM dNTPs, 5 pmol of each primer, 50-100 ng of genomic DNA and 0.5 Units of Taq DNA polymerase (Bangalore Genei, India). Amplification reactions were carried with a program as 5 min denaturation at 94°C followed by 35 cycles of 94°C for 45 s, annealing at 60°C for 45 s and extension at 72°C for 1 min, with a final extension of 10 min at 72°C. For RFLP analysis, amplified products (232 bp and 168 bp) were digested with 1 unit of *Nae*I at 37^{o} C and *Sml*I (New England Biolabs) at 55^{o} C in a final volume of 20 µl for overnight. Amplified products were separated by electrophoresis in 3% agarose in 1X TAE buffer for 90 minutes. The gel was stained with ethidium bromide, visualized under UV light, and photographed by Gel documentation system. The genetic variability was evaluated in terms of the observed number of alleles (No) and observed Heterozygosity (Hobs) for each locus and a Hardy-Weinberg (H-W) equilibrium test was performed using POPGENE software [7].

Results

Polymorphism at Prolactin receptor gene (*Nae*I)

At PRLR locus, polymorphism occurring at position 486 in Exon 10 was targeted which was a single nucleotide change (SNP), A/C (transition) which was recognizable by PCR-RFLP with *Nae*I. The PCR amplified DNA fragment of 232 bp was digested with *Nae*I restriction enzyme. *Nae*I restriction analysis of the PCR product yielded banding pattern corresponding to three different genotypes *viz*., AA genotype with one bands (232 bp), AC genotypes with three bands (232, 125 + 107 bps) and CC genotype with two bands (125 + 107 bps) shown in Fig 1. Out of 455 animals screened, 75.8% animals were homozygous AA, 3.1% of animal were homozygous CC and 21.1% animals were heterozygous (AC). The frequency of C allele was significantly lower than the A allele in all the cattle breeds.

From the allelic pattern, it was observed that A allele was predominantly present than the C allele in all the ten indigenous cattle. The frequency distribution of A allele varied from 0.787 (Kangayam) to 0.958

(Punganur) with a mean value of 0.864. The prevalence of C allele was significantly low in all the analyzed cattle breeds. The average gene frequency of C allele was estimated to be 0.136 with Kangayam showing the maximum frequency (0.213), and Punganur the minimum frequency (0.042). Thus there was preponderance of A allele compared to C allele in Zebu cattle (Table 1).

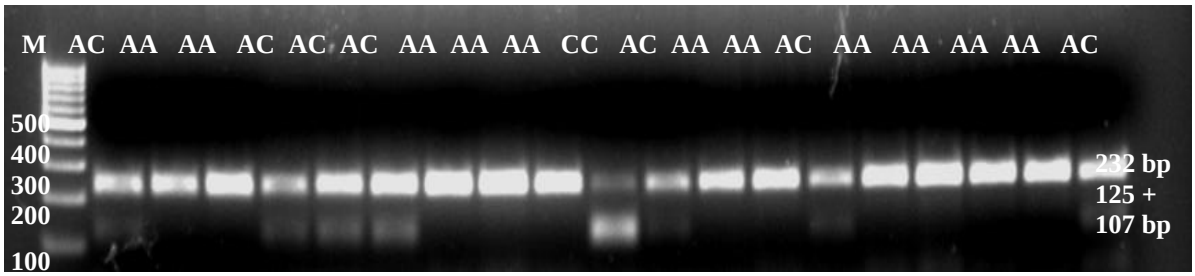

Fig. 1 Representative restriction pattern at PRLR/*Nae*I locus on 3.0% agarose gel

Table 1 No. of Animals, Observed Genotypes, Expected Genotypes, Genotypic frequency, Gene frequency and their χ^2 values of GHR-*Nae*I polymorphism in cattle

Breeds	No of Animals	Observed Genotypes			Expected Genotypes			Genotypic Frequency			Gene Frequency		
		AA	AC	CC	AA	AC	CC	AA	AC	CC	A	C	χ^2
Hariana	46	32	12	2	31.391	13.217	1.391	0.696	0.261	0.043	0.826	0.174	0.390
Kankrej	44	30	10	4	27.841	14.318	1.841	0.682	0.227	0.091	0.795	0.205	4.002
Mewati	43	35	8	0	35.372	7.256	0.372	0.814	0.186	0.000	0.907	0.093	0.452
Nagori	47	39	8	0	39.340	7.319	0.340	0.830	0.170	0.000	0.915	0.085	0.407
Tharparkar	48	42	6	0	42.188	5.625	0.188	0.875	0.125	0.000	0.938	0.063	0.213
Ghumusari	48	34	12	2	33.333	13.333	1.333	0.708	0.250	0.042	0.833	0.167	0.480
Hill Cattle	48	30	16	2	30.083	15.833	2.083	0.625	0.333	0.042	0.792	0.208	0.005
Kangayam	47	31	12	4	29.128	15.745	2.128	0.660	0.255	0.085	0.787	0.213	2.659
Binjharpuri	48	39	9	0	39.422	8.156	0.422	0.813	0.188	0.000	0.906	0.094	0.514
Punganur	36	33	3	0	33.063	2.875	0.063	0.917	0.083	0.000	0.958	0.042	0.068
Total	455	345	96	14	339.655	106.929	8.416	0.758	0.211	0.031	0.864	0.136	4.906

Polymorphism at prolactin receptor gene (*Sml*I)

At PRLR locus, polymorphisms occurred at position 828 in Exon 10. It is known to be a single nucleotide substitution (SNP), G/T (transversion) recognizable by PCR-RFLP with *Sml*I. The PCR amplified DNA fragment of 168 bp was digested with *Sml*I restriction enzyme. The various variants observed are shown in Fig 2. In the studied cattle breeds, three genotypes GG, GT and TT were observed. *Sml*I restriction analysis of the PCR product yielded banding pattern corresponding to three different genotypes *viz*., GG genotype with one bands (168 bp), GT genotypes with three bands (168, 123 and 45 bps) and TT genotype with two bands (123 and 45 bps).

Out of 454 animals screened, GT genotype was most frequent with genotypic frequency of 43.2%, followed by homozygous GG genotype with a frequency of 43.0% and TT (13.8%). The frequency of G allele was substantially high as compared to T allele in all the cattle populations. The frequency distribution of G allele across the ten breeds ranged from 0.446 (Nagori) to 0.771 (Hill Cattle) with a mean value of 0.645, whereas that of T allele ranged from 0.229 (Hill Cattle) to 0.554 (Nagori) with a mean value of 0.355 (Table2).

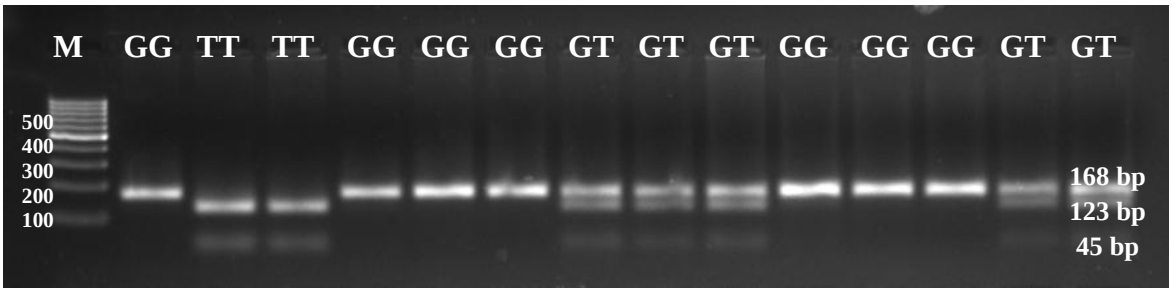

Fig. 2 Representative restriction pattern at PRLR/*Sml*I locus on 3.0% agarose gel

Table 2 No. of Animals, Observed Genotypes, Expected Genotypes, Genotypic frequency, Gene frequency and their χ^2 values of GHR-*Sml*I polymorphism in cattle

Breeds	No of Animals	Observed Genotypes			Expected Genotypes			Genotypic Frequency (%)			Gene Frequency		
		GG	GT	TT	GG	GT	TT	GG	GT	TT	G	T	χ^2
Hariana	46	19	24	3	20.891	20.217	4.891	0.413	0.522	0.065	0.674	0.326	1.610
Kankrej	44	17	27	0	21.142	18.716	4.142	0.386	0.614	0.000	0.693	0.307	8.620
Mewati	43	24	14	5	22.349	17.302	3.349	0.558	0.326	0.116	0.721	0.279	1.566
Nagori	46	10	21	15	9.136	22.728	14.136	0.217	0.457	0.326	0.446	0.554	0.266
Tharparkar	48	19	27	2	22.005	20.990	5.005	0.396	0.563	0.042	0.677	0.323	3.936
Ghumusari	47	23	17	7	21.112	20.777	5.112	0.489	0.362	0.149	0.670	0.330	1.553
Hill Cattle	48	27	20	1	28.521	16.958	2.521	0.563	0.417	0.021	0.771	0.229	1.544
Kangayam	48	18	14	16	13.021	23.958	11.021	0.375	0.292	0.333	0.521	0.479	8.293
Binjharpuri	48	25	18	5	24.083	19.833	4.083	0.521	0.375	0.104	0.708	0.292	0.410
Punganur	36	13	14	9	11.111	17.778	7.111	0.361	0.389	0.250	0.556	0.444	1.626
Total	454	195	196	63	188.876	207.909	57.215	0.43	0.432	0.138	0.645	0.355	1.466

Discussion

The genetic selection and characterization of indigenous bovine breeds assumes importance. Milk protein polymorphisms have received considerable interest because of their potential use [16]. Abdel et al., 2009 [6], utilised the DNA polymorphic markers for determination of individual genotypes at several loci for estimating population parameters such as allele frequencies and utilising them using marker assisted selection. Genotyping of milk protein and fat responsible genes such as PRLR gene is extremely

important for inclusion in selection programs to improve milk production and its constituents. Variation in PRLR is reported to be significantly associated with milk yield in Finnish Ayrshire dairy cattle [15]. Effect of PRLR polymorphism markedly influences protein and fat yield in Finnish Ayrshire dairy cattle. Moreover, the PRLR substitution is clearly associated with milk yield, protein yield, and fat yield whereas no evidence for the association of PRLR variation and milk production was found in Holstein–Friesian cattle [14]. Prolactin receptor (PRLR) is candidate gene associated with milk protein and fat yield in dairy cattle [13].

In the present study, we have established two novel PCR-RFLP loci for further studying the genetic polymorphism in cattle and their inclusion in selection programs in indigenous cattle breeds. These loci can effectively be used for genotyping PRLR gene in cattle population for association studies with milk protein and fat yield.

Conclusion

Prolactin receptor (PRLR) was investigated to characterize the variations at important gene loci and reveal their allelic status in the ten Indian cattle populations covering a large geographical area of the country. Specific alleles at different gene loci (e.g., A, C alleles at PRLR-*Nae*I and G, T alleles at PRLR-*Sml*I) were predominant with high allelic frequencies approaching fixation in Indigenous grey cattle breeds. The reported genetic variant in the GHR-exon 10 was detected. The present study argues for the involvement of some additional alleles or genes influencing milk quality and production traits with a need to investigate the Indian cattle with respect to various SNPs having associations with phenotypes of economically important traits and their utilisation in improvement programs.

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