Original Article

Polymorphism of the Prolactin Gene and Its Relationship with Milk Production in Gir and Kankrej Cattle

Jainikkumar Bipinchandra Patel, Jenabhai Bhathibhai Chauhan

Department of Genetics, Ashok and Rita Patel Institute of Integrated Studies in Biotechnology and Allied Sciences, Affiliated to Sardar Patel University, Anand, Gujarat, India

Abstract

Background: The aim of this study was to detect allelic and genotypic frequencies of prolactin (PRL) gene in Gir (200) and Kankrej (100) cattle and to analyze milk production traits of tested cattle on their PRL genotypes. **Materials and Methods:** The 156 bp fragment located in exon 3 was amplified using polymerase chain reaction-restriction fragments length polymorphism technique. **Results:** Allele frequencies in the studied breed were A = 0.52 and B = 0.48. Means and standard deviations for milk yield and fat content (%) were 3811.6 ± 462.1 and 3.99 ± 0.18 for the AA genotype, 3514.9 ± 450.8 and 4.16 ± 0.33 for the AB genotype, and 3388.8 ± 423.3 and 4.34 ± 0.11 for the BB genotype, respectively, in Gir cattle. Similarly, for Kankrej cattle, means and standard deviations for milk yield and fat content (%) were 2007.8 ± 246.3 and 4.05 ± 0.16 for the AA genotype, 1846.14 ± 133.4 and 4.10 ± 0.156 for the AB genotype, and 1767.7 ± 186.4 and 4.30 ± 0.178 for the BB genotype, respectively, in Kankrej cattle. **Conclusion:** This study showed differences in milk traits among PRL genotypes of Gir and Kankrej cattle.

Keywords: *Bos indicus*, Gir cattle, Kankrej cattle, polymerase chain reaction-restriction fragments length polymorphism, prolactin gene

Introduction

Prolactin (PRL) is one of the most multipurpose hormones of the pituitary gland in terms of biological actions. PRL is a polypeptide hormone that is synthesized and secreted from specialized cells of the anterior pituitary gland, and more than 100 different and distinct effects of the hormone have been documented so far.[1] PRL plays extremely important roles in the growth and development of the mammary gland (mammogenesis), maintenance of milk secretion (galactopoiesis), synthesis of milk (lactogenesis), which affecting milk yield and composition.[2] It is also primarily responsible for the synthesis of lactose, lipids and all other major components of milk.[3] Therefore, the gene encoding PRL is considered one of the most important key links in the gene network constituting the hereditary component of milk productivity. These characteristics make PRL gene a strong candidate gene for milk traits.

The PRL gene has been mapped to chromosome 23 at 43 cM close to the quantitative trait loci (QTL) in bovine,[4] about 10 kb in size, includes 5 exons coding for 199 amino acids and 4 introns.[5] The silent A→G (adenosine-guanine) transition mutation at the codon for amino acid 103 in exons 3 of bovine PRL gene has been reported to give rise to a polymorphic site for *Rsa*I restrictions enzyme.[6] It has become a popular genetic marker used for the genetic characterization of *Bos indicus* cattle populations by means of polymerase chain reaction-restriction fragments length polymorphism (PCR-RFLP).[7-10]

There are many hormones that regulate lactation and reproduction in bovine, PRL is one of the important anterior pituitary hormones. The milk protein and hormone genes are excellent candidate genes for linkage analysis with QTL. The aim of this study is to determine the relationship between different genotypes of PRL gene and its effect on milk traits in Gir and Kankrej indigenous cattle breed.

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Address for correspondence: Dr. Jenabhai Bhathibhai Chauhan, Department of Genetics, Ashok and Rita Patel Institute of Integrated Studies in Biotechnology and Allied Sciences, Affiliated to Sardar Patel University, New Vallabh Vidyanagar, Anand - 388 121, Gujarat, India.
E-mail: jenabhaichauhan@aribas.edu.in

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Materials and Methods

Experimental materials

Experimental material for this study comprised blood samples from 278 Gir and 97 Kankrej cattle. The blood samples were collected from the jugular vein of cattle into potassium ethylenediaminetetraacetic acid containing vacutainer (BD Vacutainer). All the animals were unrelated and selected at random from the herds, maintained at Kankrej cattle breeding farm Thara and Mandvi of Gujarat Livestock Development Board, Livestock Research Station, College of Veterinary Science and Animal Husbandry cattle farm (AAU), Anand, private cattle farm of Anand and Kheda district of Gujarat. The Gir (22) and Kankrej (3) cattle semen straw were received from Amul Research and Development Association, Ode farm and Sabarmati Ashram Gaushala, Bidaj farm. The blood and semen samples were transported to the laboratory in ice box at 4°C. The milk traits recorded or maintained in the farm were 305 days correct milk yield and average milk fat yield.

DNA isolation

Genomic DNA was extracted by phenol-chloroform method as described by John *et al*.,[11] with minor modifications. The quality of isolated genomic DNA was checked electrophoretically using 0.8% agarose gel, whereas the quantity of isolated genomic DNA was checked by NanoDrop spectrophotometer (Thermo scientific™ Nanodrop 2000).

Polymerase chain reaction-restriction fragments length polymorphism analysis

A pair of PRL gene exon 3 specific primers (forward - 5' CGA GTC CTT ATG AGC TTG ATT CTT 3' and reverse - 5' GCC TTC CAG AAG TCG TTT GTT TTC 3') was used for amplification.[7] PCR was carried out in a final reaction volume of 25 µl; each reaction contained 200 µM of each dNTP, 10 pM of each primer, 1 unit of *Taq* DNA polymerase and 100 ng of template DNA in 1X PCR buffer. Amplification cycling conditions were 94°C for 5 min followed by thirty cycles comprising denaturation at 94°C for 1 min, annealing at 59°C for 40 s and extension at 72°C for 20 s, followed by a final extension step at 72°C for 5 min. The PCR reaction products was electrophoresed on 1.5% agarose gel and stained with ethidium bromide to detect the amplification success. The PCR products were digested with 5 units of *Rsa*I (New England Biolabs) at 37°C for 1 h in a final reaction volume of 25 µl. After restriction digestion, the restricted fragments were analyzed electrophoretically using 3% agarose gel, stained with ethidium bromide. The 100 bp ladder was used as molecular size marker. The bands were visualized under ultraviolet light and the gels were photographed using camera.

Statistical analysis

Genotyping and allele frequency of PRL was calculated by simple, direct allele counting. The Hardy–Weinberg equilibrium (HWE) and Chi-square test was used to determine the possible deviations of genotype frequencies from expectation.[12] The effect of PRL genotype on milk production traits in standard length of lactation was analyzed and the significant differences were determined using one-way analysis of variance. The data analysis for this paper was generated using the Real Statistics Resource Pack software (Release 4.3). Copyright (2013 – 2015) Charles Zaiontz. www.realstatistics.com.[13] The relationship between genotypes and traits was considered statistically significant when $P < 0.05$.

Results and Discussion

In the PRL gene point mutation from adenine-guanine (A103G), that results in two alleles A and B. The allele adenine that has at position 103 was denoted as the A allele. The mutation from adenine-guanine caused the formation of a restriction site for *Rsa*I restriction enzyme and this allele was denoted as B allele. The 156 bp fragment of exon 3 region of PRL gene was amplified, digested with *Rsa*I restriction endonuclease, and separated electrophoretically using 3% agarose gel which revealed three genotypic patterns across two (Gir and Kankrej) Indian zebu cattle breeds. The first pattern with an uncut single fragment of 156 bp was designated the AA genotype (absence of restriction site). The second pattern with two fragments (82 and 74 bp) was referred to as BB (presence of restriction site); if both of the gene copies had restriction site, then two fragments was expected. The third pattern with three fragments (156, 82 and 74 bp) was AB genotype; if just one of the gene copies had restriction site, then three fragments were expected [Figure 1].

The HWE was investigated with Chi-square test;[12] allele frequencies, observed and expected genotypes, and *P*-value of PRL gene are summarized in Table 1. The value of Chi-square for Gir cattle was 13.46, higher than the critical value of 5.99, which means that the population under study is not in HWE, $P = 0.00119$, which is statistically significant at $P < 0.05$. Similarly, for Kankrej cattle, Chi-square value was 12.58, higher than the critical value of 5.99, which means that the population under study is not in HWE, $P = 0.00185$, which is statistically significant at $P < 0.05$.

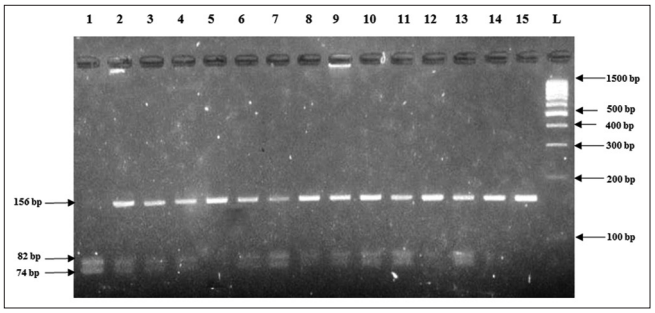

Figure 1: Electrophoretic pattern of *Rsa*I digested polymerase chain reaction product of representative samples on 3% agarose, in Lane 1, two fragments with 82 and 74 bp (BB genotype), in Lane 5, 12, and 14 one uncut fragment with 156 bp, in Lane 2, 3, 4, 6, 7, 8, 9, 10, 11, 13 three fragments with 156, 82 and 74 bp (AB genotype), in Lane 15-polymerase chain reaction product, in Lane 16 low-range DNA ladder (100 bp)

The allelic profile data of PRL gene showed similar gene frequencies for A and B alleles of both Gir and Kankrej cattle, with mean values of 0.52 and 0.48, respectively. The PRL gene genotype variants obtained through the PCR-RFLP technique with the *Rsa*I endonuclease, the heterozygous AB genotype (for Gir cattle 0.83 while Kankrej cattle 0.70) had the greatest frequency, which is similar to the data obtained by several researchers in different regions of India, with different sample sizes and breeds, who had reported genotype frequencies from 0.49 to 0.62, in Kankrej (0.62), Gir (0.49), and Red Sindhi (0.62).[9] Similar result was found by Sodhi *et al*. in their study, who reported a higher frequency of AB genotype (0.77) of PRL gene for Gir cattle which was almost equal to presently observed (0.83).[8]

The effect of the PRL gene in milk yield (from the available pedigree data) showed the best mean value for the genotype AA with 3811.6 kg, followed by AB with 3514.9 and BB with 3388.8 kg in Gir cattle breed [Table 2]. A similar result was also found in this study where Kankrej cattle with AA genotype cattle produced 2007.8 kg, followed by AB genotype cattle produced 1846.1 kg and BB genotype cattle produced 1767.7 kg milk. There was a significant difference between genotype AA and the other two (AB and BB genotype), thus determining the influence of the A allele in milk yield of both Gir and Kankrej cattle (*B. indicus*) ($P < 0.05$). The BB genotype Gir and Kankrej cattle have higher fat percentage as compared to AA and AB genotype cattle [Table 2].

When analyzing the genotypes favorable for milk production in *B. indicus* cattle, it was found that the genotype AA had the best average with 3811.6 kg in Gir cattle breed while 2007.8 kg in Kankrej cattle breed. This result is similar for Montebeliard cattle in which AA genotype cattle produced 5805 kg milk.[14] Chung *et al*.[15] reported that Holstein–Friesian cows with AA genotype produced milk with higher fat content than BB individuals. Dybus *et al*.[16] found that the genotype AA was favorable for the second and third lactations whereas the genotype AB was in the first lactation in Jersey cattle and both genotypes AA and AB were favorable in Black and White cattle.

Conclusion

According to the results obtained in this study, it is concluded that the most frequent genotype in Gir and Kankrej cattle population was AB (0.786). Based on the statistical analysis, AA genotypes may have greater influence on average milk yield ($P < 0.05$) among Gir and Kankrej cattle. Our findings revealed that significant difference was also found among cattle with different PRL gene genotypes (AA, AB and BB) in terms of average fat percentage ($P < 0.05$). According to the current results, cattle with AA genotype were associated with higher milk yield and less fat percentages as compared to cattle of other genotypes. The reasons may be due to the negative correlation between milk yield and fat percentages in cattle. Finally, allele A of PRL can be considered as a good indicator for milk production in the Gir and Kankrej cattle breed. The research on PRL gene polymorphism should be extended to cattle population of different geographical regions of Gujarat to confirm the influence of PRL/*Rsa*I marker on milk production traits. Such results may be used as a selection criterion to facilitate genetic selection in cattle breeding programs.

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Table 1: Allele frequencies and result of Chi-square test for comparison of proportions between Gir and Kankrej cattle

Cattle	*n*	∑	Prolactin genotype			Alleles frequencies		Chi-square test	*P*
			AA	AB	BB	A	B		
Gir	200	Observed	25	166	09	0.54	0.46	13.46	0.00119* (df=2)
		Expected	25.33	157.33	17.33				
Kankrej	100	Observed	13	70	17	0.48	0.52	12.58	0.00185* (df=2)
		Expected	12.66	78.66	8.66				
Total	300	Observed	38	236	26	0.52	0.48	26.04	0.00304* (df=2)

*Significant at $P<0.05$. df: Degree of freedom

Table 2: Means and standard deviations of milk production traits in Gir and Kankrej cattle of different prolactin gene genotypes

Breed	Genotypes	*n*	Traits (means±SD)	
			Mean milk yield (kg/305 days)	Fat content percentage
Gir	AA	9	3811.6±462.13[a]	3.99±0.18
	AB	166	3514.9±450.81[a]	4.16±0.33
	BB	25	3388.8±423.36[a]	4.34±0.11
Kankrej	AA	13	2007.8±246.3[a]	4.05±0.16
	AB	70	1846.1±133.4[a]	4.10±0.156
	BB	17	1767.7±186.4[a]	4.30±0.178

[a]Significant difference at $P<0.05$. SD: Standard deviation

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Conflicts of interest

There are no conflicts of interest.

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