Biochem Genet (2011) 49:39–45
DOI 10.1007/s10528-010-9383-7

Analysis of Genetic Variation at the Prolactin-*Rsa*I (*PRL-Rsa*I) Locus in Indian Native Cattle Breeds (*Bos indicus*)

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Received: 10 February 2010 / Accepted: 24 June 2010 / Published online: 29 September 2010

Abstract This study assessed the distribution pattern of allelic variants at the prolactin-*Rsa*I locus in 23 Indian native cattle breeds (*Bos indicus*). PCR–RFLP genotyping of a 156 bp fragment of prolactin (PRL) in exon 3 revealed the predominance of the heterozygous AB genotype (mean frequency 0.58) irrespective of utility type (dairy, dual, draft), geographic region (northern, central, southern), and coat color (red, gray) of the breeds analyzed. The overall frequencies of homozygous AA (0.22) and BB (0.20) genotypes were in a similar range. The PRL^{A} and PRL^{B} alleles exhibited similar gene frequencies (means 0.52 and 0.48, respectively). The existing profile of the *PRL-Rsa*I gene locus in a large set of Indian native cattle breeds was different from that of *Bos taurus* and cattle breeds of other countries, where either the BB genotype and PRL^{B} allele or the AA genotype and PRL^{A} allele have been reported to be more prevalent.

Keywords Indian native cattle · Prolactin gene · Allelic variation · PCR–RFLP

Introduction

Prolactin (PRL) is one of the most versatile polypeptide hormones of the pituitary gland, with more than 300 activities reported in various classes of vertebrates (Bole-Feysot et al. 1998). It is an important regulator of mammary growth and lactogenesis (Wallis 1974; Collier et al. 1984) and acts on mammary alveoli to promote the synthesis and secretion of milk proteins in bovines (Le Provost et al. 1994). It also regulates reproductive and immunological functions, fluid balance, and cellular growth and differentiation (Nicoll 1980; Loretz and Bern 1982; Russell

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1989). Gene expression has also been observed at several other sites, besides the pituitary gland, including the central nervous system, the immune system, and the mammary gland (Sinha 1995; Ben-Jonathan et al. 1996; Le Provost et al. 1994). The multifaceted functional importance of the bovine *PRL* gene makes it an important genetic marker for quantitative traits.

The bovine *PRL* gene, localized in chromosome 23 (Barendse et al. 1997), consists of five exons coding for 199 amino acids and four introns (Camper et al. 1984). A silent A–G transition mutation at amino acid 103 in exon 3 of this gene has been reported to give rise to a polymorphic *Rsa*I site (Lewin et al. 1992). In the past, several groups have described *PRL-Rsa*I polymorphism in cattle breeds from various countries and evaluated their association with milk yield, fat percentage, and milk protein content (Lewin et al. 1992; Golijow et al. 1999; Udina et al. 2001; Dybus et al. 2005; Ghasemi et al. 2009).

No systematic effort, however, has been undertaken to assess the status of *PRL-Rsa*I polymorphism (gene frequency, gene diversity, and differences between breeds) in diverse Indian native cattle (*Bos indicus*) breeds. As a consequence of evolutionary divergence, these Indian zebu cattle might possess allelic combinations different from the highly selective taurine cattle. Further, being naturally evolved and adapted to the tropical climate, these become an important resource for characterization and genetic evaluation studies. This study was therefore designed to characterize allelic diversity at the *PRL-Rsa*I gene locus in a large set of Indian cattle.

Materials and Methods

We genotyped 938 unrelated DNA samples representing 23 Indian native cattle breeds of divergent types based on their utility, coat color, agroclimatic zone, and geographic location (Table 1) to uncover the status of *PRL-Rsa*I locus polymorphism. Breeds were categorized based on their utility for dairy, draft, or dual purposes. When categorized by coat color, the majority of the breeds were of the gray type (15) and the rest were of the red/brown type (8). Breeds were divided into three geographic groups: from the north/northwest region including Haryana, Punjab, Rajasthan, and Gujarat states; from the central region representing Madhya Pradesh and Maharashtra states; and from a southern region covering Andhra Pradesh, Tamil Nadu, and Karnataka states. The four agroclimatic zones were tropical wet and dry, semiarid, arid, and humid subtropical. Blood samples were collected randomly from the respective breeding tracts of the breeds. Genomic DNA was extracted using the standard protocol of Sambrook et al. (1989).

PCR–RFLP Based Genotyping of the *PRL-Rsa*I Locus

A fragment of 156 base pairs (bp) of the *PRL* gene at exon 3 was amplified using the primer pair (forward 5′-CGAGTCCTTATGAGCTTGATTCTT-3′ and reverse 5′-GCCTTCCAGAAGTCGTTTGTTTTC-3′) reported by Lewin et al. (1992). The PCR reaction was carried out in a reaction volume of 25 μl containing 100 ng

Table 1 Characteristics of 23 Indian zebu cattle breeds included in this study

Breed	Utility type	Geographic location	Coat color	Agroclimatic zone
Amritmahal	Draft	South	Gray/white	Tropical wet and dry
Dangi	Draft	Central	Gray/white	Tropical wet and dry
Deoni	Dual	Central	Gray/white	Tropical wet and dry
Gaolao	Dual	Central	Gray/white	Humid subtropical
Gir	Dairy	Northwest	Red	Semiarid
Hariana	Dual	North	Gray/white	Semiarid
Kangayam	Draft	South	Gray/white	Semiarid
Kankrej	Dual	Northwest	Gray/white	Semiarid
Kherigarh	Draft	Central	Gray/white	Humid subtropical
Khillar	Draft	Central	Gray/white	Tropical wet and dry
Malnad Gidda	Draft	South	Red/brown	Humid subtropical
Malvi	Draft	Central	Gray	Semiarid
Mewati	Dual	North	Gray/white	Semiarid
Nagori	Draft	Northwest	Gray/white	Arid
Nimari	Draft	Central	Red	Tropical wet and dry
Ongole	Dual	South	Gray/white	Tropical wet and dry
Ponwar	Draft	Central	Red/brown	Humid subtropical
Rathi	Dairy	Northwest	Red/brown	Arid
Red Kandhari	Draft	Central	Red	Tropical wet and dry
Red Sindhi	Dairy	Northwest	Red	Arid
Sahiwal	Dairy	North/Northwest	Red	Semiarid
Tharparkar	Dairy	Northwest	Gray/white	Arid
Umblachery	Draft	South	Gray	Tropical wet and dry

genomic DNA, 5 pmol each primer, 1.5 mM $MgCl_2$, 200 μM dNTP, and 1.0 U *Taq* polymerase (Invitrogen, USA). PCR was carried out with a PTC 100 apparatus (MJ Research, MA, USA). Amplification cycling conditions were 95°C for 5 min; followed by 30 cycles at 94°C for 30 s, 59°C for 40 s, and 72°C for 20 s; with a final extension at 72°C for 3 min. For the PCR–RFLP analysis, 10 μl each PCR amplified product was digested with 2 U *Rsa*I enzyme (MBI Fermentas, USA) at 37°C for 4 h followed by 20 min of inactivation at 65°C. The digestion products were separated on 3.5% agarose gel in 1× TAE buffer by electrophoresing at 80 volts, 25 min. The number of DNA samples utilized for PCR–RFLP analysis from each breed ranged from 26 to 48 (Table 2).

Statistical Analysis

Genotype frequency was calculated based on the direct gene count method using the formula $(n_{AB} + 2n_{BB})/2n$. A chi square test was used to evaluate the significance of differences in allele and genotype frequency distribution among groups based on their utility pattern, coat color, or location.

Table 2 Allele and genotype frequency at the *PRL-Rsa*1 locus in 23 Indian native cattle breeds

Breed	*N*	Allele frequency		Genotype frequency		
		A	B	AA	AB	BB
Amritmahal	46	0.70	0.30	0.46	0.48	0.06
Dangi	36	0.39	0.61	0.14	0.50	0.36
Deoni	30	0.45	0.55	0.23	0.43	0.34
Gaolao	40	0.62	0.38	0.53	0.20	0.27
Gir	47	0.62	0.38	0.23	0.77	0.00
Hariana	46	0.41	0.59	0.27	0.30	0.43
Kangayam	48	0.55	0.45	0.17	0.77	0.06
Kankrej	33	0.38	0.62	0.18	0.40	0.42
Kherigarh	26	0.58	0.42	0.19	0.77	0.04
Khillar	38	0.55	0.45	0.21	0.69	0.10
Malnad Gidda	44	0.54	0.46	0.14	0.64	0.23
Malvi	44	0.40	0.60	0.18	0.43	0.39
Mewati	47	0.50	0.50	0.02	0.96	0.02
Nagori	37	0.50	0.50	0.22	0.57	0.21
Nimari	46	0.65	0.35	0.43	0.44	0.13
Ongole	37	0.51	0.49	0.32	0.38	0.30
Ponwar	29	0.45	0.55	0.10	0.69	0.21
Rathi	45	0.50	0.50	0.16	0.67	0.17
Red Sindhi	41	0.54	0.46	0.22	0.63	0.15
Red Kandhari	47	0.55	0.45	0.28	0.55	0.17
Sahiwal	41	0.51	0.49	0.24	0.54	0.22
Tharparkar	46	0.51	0.49	0.07	0.89	0.04
Umblachery	44	0.47	0.53	0.09	0.75	0.16
Mean		0.52 ± 0.083	0.48 ± 0.083	0.22 ± 0.122	0.58 ± 0.190	0.20 ± 0.131

N, number of DNA samples (total 938)

Results and Discussion

Restriction digestion analysis of the 156 bp PCR product of the exon 3 region of the *PRL* gene with *Rsa*I enzyme revealed three genotypic patterns across 23 Indian cattle breeds. The first pattern, with a single fragment of 156 bp, was designated the AA genotype (lacking restriction site). The second pattern, with two fragments (82 and 74 bp), was referred to as BB, and the third pattern, with three fragments (156, 82, and 74 bp) was the AB genotype (Fig. 1; Table 2).

Of the three genotypes, the heterozygous AB genotype was present in the highest frequency across all the breeds, irrespective of their utility type (dairy, dual, draft), geographic region (north, central, south), and coat color (red and gray). The frequency of the AB genotype ranged from 0.20 (Gaolao) to 0.96 (Mewati), with a mean of 0.58. The two homozygous genotypes, AA and BB, showed similar mean frequencies of 0.22 and 0.20, respectively. The allelic profile data showed similar

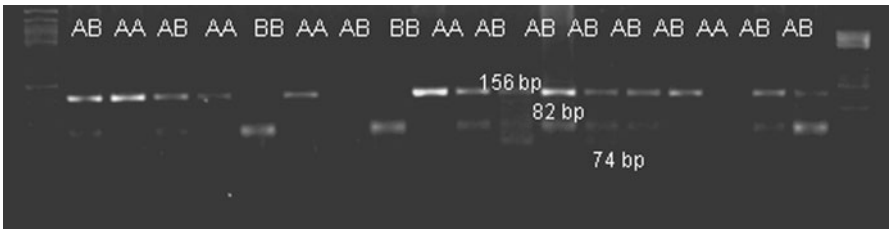
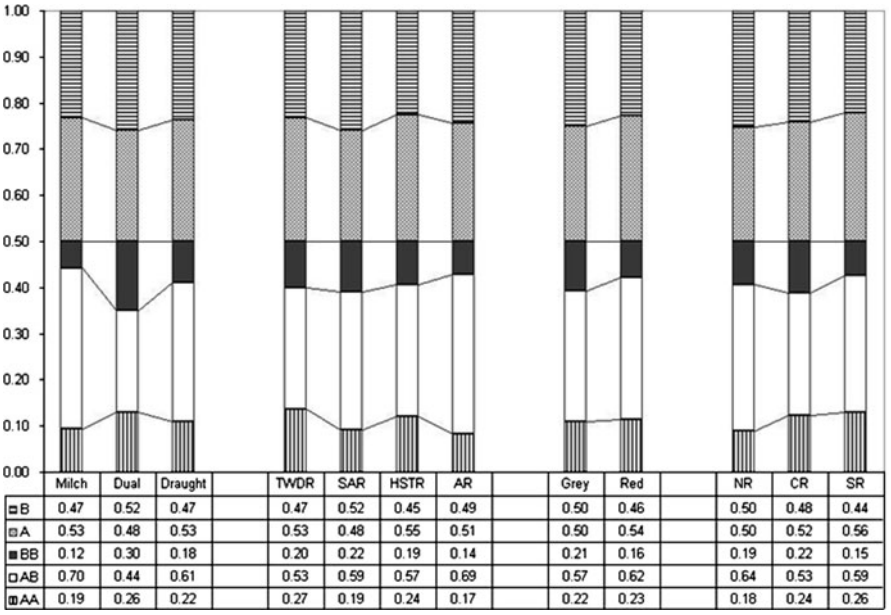


Fig. 1 Representative restriction pattern at the *PRL* locus, on 3.5% agarose gel. AA genotype: 156 bp fragment. AB genotype: 156, 82, and 74 bp fragments. BB genotype: 82 and 74 bp fragments. Molecular weight standard from Roche Applied Science; 50 bp ladder (MBI)

gene frequencies for the PRL^A and PRL^B alleles, with a mean value of 0.52 and 0.48, respectively. Amritmahal and Kankrej cattle showed the highest frequencies of PRL^A (0.70) and PRL^B (0.62), respectively.

To understand the frequency pattern across the types of cattle, breeds were grouped on the basis of their utility type, coat color, geographic location, and agroclimatic region. Overall, the allelic (PRL^A and PRL^B) and genotypic (AA, AB, and BB) profiles (Fig. 2) of the categories of breeds showed nonsignificant differences using the chi square test ($P > 0.05$, data not shown).



	Milch	Dual	Draught	TWDR	SAR	HSTR	AR	Grey	Red	NR	CR	SR
B	0.47	0.52	0.47	0.47	0.52	0.45	0.49	0.50	0.46	0.50	0.48	0.44
A	0.53	0.48	0.53	0.53	0.48	0.55	0.51	0.50	0.54	0.50	0.52	0.56
BB	0.12	0.30	0.18	0.20	0.22	0.19	0.14	0.21	0.16	0.19	0.22	0.15
AB	0.70	0.44	0.61	0.53	0.59	0.57	0.69	0.57	0.62	0.64	0.53	0.59
AA	0.19	0.26	0.22	0.27	0.19	0.24	0.17	0.22	0.23	0.18	0.24	0.26

Fig. 2 Mean allele and genotype frequencies of *PRL-Rsa*I in Indian cattle breeds on the basis of four characteristics. *Top bars*: *horizontal stripe* allele B, *gray* allele A. *Bottom bars*: *black* genotype BB, *white* genotype AB, *vertical stripe* genotype AA. Characteristics, *left to right*: utility (dairy, dual purpose, draft); agroclimatic region (tropical wet and dry, semiarid, humid subtropical, arid); coat color (gray, red), and geographic location (north, central, south)

Contrary to the present observations (PRL^A frequency 0.52), several studies have reported a higher frequency of the PRL^A allele in European Holstein (0.95, Chrenek et al. 1998), Korean Holstein (0.73, Chung et al. 1996; 0.89, Jang et al. 2005), Russian Ayrshire (0.86, Udina et al. 2001), and Gorbatev Red (0.91, Udina et al. 2001) cattle. Studies on Korean Holstein (Chung et al. 1996; Jang et al. 2005) and two Russian cattle (Udina et al. 2001) showed a complete absence of the homozygous BB genotype in these breeds. Dybus et al. (2005) also presented a higher frequency of AA genotypes in two herds of Black (0.722) and White Polish (0.698) cattle. These studies thus confirmed the predominance of the PRL^A allele in taurine cattle breeds. Conversely, our data set revealed a similar distribution of the two alleles in Indian cattle breeds. A higher mean frequency (0.48) of the PRL^B allele in Indian cattle corroborated the findings of Mitra et al. (1995) and Sacravarty et al. (2008), who also observed an equal prevalence of PRL^A and PRL^B alleles in Sahiwal and Kankrej cattle.

This study thus revealed a distribution of *PRL-Rsa*I variants in Indian cattle distinct from that of taurine cattle, which might be attributed to the genomic difference between the two cattle types. The difference in allelic pattern between the two cattle types has also been reported at the *Msp*I-GH (Sodhi et al. 2007) and the A1/A2 β-casein (Mishra et al. 2009) gene loci. As Indian cattle present unique allelic patterns at several loci, they constitute an interesting resource for finding new genetic variations. Further, considering that prolactin is a multipurpose hormone influencing a number of traits, polymorphisms in the regulatory region and their linkages with the *PRL-Rsa*I variants would be of great significance for genotype–phenotype association studies in Indian cattle.

Acknowledgment This work received financial support from the Indian Council of Agriculture Research (ICAR, New Delhi).

References

Barendse W, Vaiman D, Kemp SJ, Sugimoto Y, Armitage SM, Williams JL, Sun HS, Eggen A, Agaba M, Band M, Bishop MD, Buitkamp J, Byrne K, Collins F, Cooper L, Coppieters W, Denys B, Drinkwater RD (1997) A medium-density genetic linkage map of the bovine genome. Mamm Genome 8:21–28

Ben-Jonathan N, Mershon JL, Allen DI, Steinmetz RW (1996) Extra pituitary prolactin, distribution, regulation, functions, and clinical aspects. Endocr Rev 17:639–669

Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA (1998) Prolactin (PRL) and its receptor, actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev 19:225–268

Camper SA, Luck DN, Yao Y, Woychik RP, Goodwin RG, Lyons RH, Rootman FM (1984) Characterization of the bovine prolactin gene. DNA 3:237–249

Chrenek P, Vasicek D, Bauerovf M, Bulla J (1998) Simultaneous analysis of bovine growth hormone and prolactin alleles by multiplex PCR and RFLP. Czech J Anim Sci 43:53–55

Chung ER, Rhim TJ, Han SK (1996) Associations between PCR–RFLP markers of growth hormone and prolactin genes and production traits in dairy cattle. Korean J Anim Sci 38:321–336

Collier RJ, McNamara JP, Wallace CR, Dehoff MH (1984) A review of endocrine regulation of metabolism during lactation. J Anim Sci 59:498–510

Dybus A, Grzesiak W, Kamieniecki H, Szatkowska I, Sobek Z, Błaszczyk P, Czerniawska-Piatkowska E, Zych S, Muszynska M (2005) Association of genetic variants of bovine prolactin with milk production traits of Black-and-White and Jersey cattle. Arch Tierz 48:149–156

Ghasemi N, Zadehrahmani M, Rahimi G, Hafezian SH (2009) Associations between prolactin gene polymorphism and milk production in montebeliard cows. Int J Genet Mol Biol 1(3):048–051

Golijow CD, Giovambattista G, Rípoli MV, Dulout FN, Lojo MM (1999) Genetic variability in Argentine Creole and Argentine Holstein cattle. Genet Mol Biol 22(3):395–398

Jang GW, Cho KH, Kim TH, Oh SJ, Cheong IC, Lee KJ (2005) Association of candidate genes with production traits in Korean dairy proven and young bulls. Asian Aust J Anim Sci 18(2):165–169

Le Provost F, Leroux C, Martin P, Gaye P, Digane J (1994) Prolactin gene expression in ovine and caprine mammary gland. Neuroendocrinology 60:305–313

Lewin HA, Schmitt K, Hubert R, Van Eijk MJ, Arnheim N (1992) Close linkage between bovine prolactin and *BoLA-DRB3* genes, genetic mapping in cattle by single sperm typing. Genomics 13:44–48

Loretz CA, Bern HA (1982) Prolactin and osmoregulation in vertebrates. Neuroendocrinology 35:292–304

Mishra BP, Mukesh M, Prakash B, Sodhi M, Kapila R, Kishore A, Kataria RR, Joshi BK, Bhasin V, Rasool TJ, Bujarbaruah KM (2009) Status of milk protein, β-casein variants among Indian milch animals. Indian J Anim Sci 79:722–725

Mitra A, Schlee P, Balakrishnan CR, Pirchner F (1995) Polymorphism at growth-hormone and prolactin loci in Indian cattle and buffalo. J Anim Breed Genet 112:71–74

Nicoll CS (1980) Ontogeny and evolution of prolactin's functions. FASEB J 39:2563–2566

Russell DH (1989) New aspects of prolactin and immunity: a lymphocyte-derived prolactin-like product and nuclear protein kinase C activation. Trends Pharmacol Sci 10:40–44

Sacravarty G, Vadodaria VP, Joshi CG, Brahmkhtri BP, Shah RR, Solanki JV (2008) Prolactin gene polymorphism and its association with economic traits in Kankerj cattle. Indian J Dairy Sci 61(4):273–276

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York

Sinha YN (1995) Structural variants of prolactin, occurrence and physiological significance. Endocr Rev 16:354–369

Sodhi M, Mukesh M, Prakash B, Mishra BP, Sobti RC, Singh KP, Singh S, Ahlawat SPS (2007) *Msp*I allelic pattern of bovine growth hormone (bGH) gene in Indian zebu cattle (*Bos indicus*) breeds. Biochem Genet 45(1–2):145–153

Udina IG, Turkova SO, Kostyuchenko MV, Lebedeva LA, Sulimova GE (2001) Polymorphism of bovine prolactin gene, microsatellite, PCR–RFLP. Russ J Genet 37(4):407–411

Wallis M (1974) The primary structure of bovine prolactin. Fed Eur Biochem Soc Lett 44:205–208