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**Ruksana M Shah**
Assistant Prof. Division of AGB, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

**NA Ganai**
Division of AGB, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

**FD Sheikh**
Division of AGB, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

**S Shanaz**
Division of AGB, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

**HM Khan**
Division of LPM, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

**Safeer Alam**
Division of AGB, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

**Nusrat N Khan**
Division of AGB, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

**Tavsief Sheikh**
Division of AGB, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

**Saba Bukhari**
Division of AGB, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

**Ambreen Hamadani**
Division of AGB, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

**Mubashir A Rather**
SDO, Budgam, Jammu and Kashmir, India

**Corresponding Author:**
**Ruksana M Shah**
Assistant Prof. Division of AGB, FVSc & AH, SKUAST-Kashmir, Jammu and Kashmir, India

# Exon IV prolactin (PRL) gene polymorphism and its association with milk production traits in dairy cattle of Kashmir, India

**Ruksana M Shah, NA Ganai, FD Sheikh, S Shanaz, HM Khan, Safeer Alam, Nusrat N Khan, Tavsief Sheikh, Saba Bukhari, Ambreen Hamadani and Mubashir A Rather**

**Abstract**
Prolactin plays an important regulatory function in mammary gland development, milk secretion, and expression of milk protein genes. Hence the PRL gene is a potential quantitative trait locus and genetic marker of production traits in dairy cattle. We analysed the best genotypes of Exon IV of PRL gene influencing the yield and quality of milk. 120 Jersey and Crossbred HF cows (60 each) were phenotyped for milk yield and quality traits. Genomic DNA was extracted using Phenol-chloroform method. 50ml morning milk samples were collected on weekly basis and analysed for quality using Speedy Lab Milk Auto-analyser. SAS 9.3 Statistical software was used for association analysis. The PRL gene (294 bp) was screened for polymorphisms by PCR-RFLP using *Rsa I* enzyme yielded three genotypes (RR, Rr and rr) for Crossbred cows and two genotypes (RR and Rr) in Jersey cows. The frequency of allele "R" was 0.50 in Crossbred HF cows and high in Jersey cows (0.70). In Crossbred HF cows genotype RR showed significant ($p$<0.05) differences on all the yield traits under study whereas it was genotype Rr found to be responsible for higher milk yield traits in Jersey cows. Genotypic effect was found to be significant ($p$<0.05) on protein%, fat% and density in Crossbred HF cows whereas in Jersey cows effect was non-significant ($p$>0.05) on all quality traits.

**Keywords:** prolactin gene, exon IV, genotypes, milk yield and quality

## 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, so far distinct effects of the hormone have been documented [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) affecting milk yield and its 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]. 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 [6-9]. Keeping in view the importance of PRL gene in milk production the study was aimed to determine the relationship between different genotypes of PRL gene Exon IV and its effect on milk traits in Jersey and HF crossbred cows of Kashmir, India.

## Materials and Methods
### Experimental materials
The study was undertaken on 120 dairy cows of two genetic groups Jersey and Crossbred HF cows (60 each) belonging to an organized farm MLRI, SKUAST-K. Daily milk records and milk samples (50ml) each were collected in sterile containers and were analysed for quality using Speedy Lab Milk Auto-analyser for the Fat, Protein, SNF, Lactose, Density and Ash parameters.

**Blood samples, DNA extraction, purification and quantification**

10 ml of blood was collected from jugular vein of each animal in a 15-ml sterile graduated polypropylene tubes containing EDTA (0.5 M, pH=8.0). Genomic DNA was isolated by standard phenol-chloroform extraction method [10]. The quality and quantity of the genomic DNA were determined by measuring the absorbance at 260mm for DNA concentration and 260/280mm for DNA purity using spectrophotometer. Working dilution of extracted DNA was prepared for each sample at a concentration of 50 ng/µl.

**PCR amplification and RFLP**

Primer pair forward 5´CCAAATCCACTGAATTATGCTT 3´ and reverse 5´ACAGAAATCACCTCTCTCATTCA3´ were used for amplification of Exon IV (294 bp) [11]. PCR was carried out in a final reaction volume of 25 µl; each reaction contained 200 µM of each dNTP, 50 ng of each primer, 1 unit of *Taq* DNA polymerase and 500 ng of template DNA in 10X buffer with $MgCl_2$. Amplification cycling conditions were 94°C for 2 min followed by thirty-six cycles comprising denaturation at 94°C for 30 secs, annealing at 57°C for 30 secs and extension at 72°C for 45 secs, followed by a final extension step at 72°C for 10 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 (Thermo Scientific) at 37°C for 1 h in a final reaction volume of 25 µl. After restriction digestion, the restricted fragments were analysed electrophoretically using 3% agarose gel, stained with ethidium bromide. The digested products were visualized under UV light on transilluminator. The banding patterns were scored manually, and gels was recorded in a Gel Documentation System.

*Statistical analysis and association study*

The frequency of different genotypes and alleles were calculated by using Popgene 1.31 statistical software [12]. To analyse the Hardy-Weinberg equilibrium of the population software Popgene version= 1.31 [12] was used. The following general linear model was used for obtaining the association followed by Tukey's test using SAS 9.3 statistical software.

$$Y_{ijk} = \mu + B_j + Gk + E_{ijk}$$

Where $Y_{ijk}$ is the observation of $i^{th}$ animal of $j^{th}$ breed with $k^{th}$ genotype, $Y_{ijk}$ is the milk yield/fat yield/ protein yield/ SNF/density/ash/lactose, µ the overall mean, Bj equals 1-2 (Jersey and Crossbred HF), Gk is the 1-3 (genotypes for selected genes), and $E_{ijk}$ is the random error.

**Results and Discussion**

**PCR amplification and polymorphism analysis**

A specific single band of lengths 294 bp was amplified in both the genetic groups (Plate 1). Exon IV region (294 bp) on digestion revealed three banding pattern and were designated as RR (presence of single band of 294bp), Rr (presence of three bands 294 bp, 162bp and 132bp) and rr (presence of two bands of 162 bp and 132bp). In Crossbred HF cows, all the three patterns were present but in Jersey cows genotype rr was absent (Plate 2). Similar RFLP patterns using *Rsa* I restriction enzyme were reported in exotic and Zebu cattle [7], Kankrej cattle [8] and in Montebeliard cows [12].

**Gene and genotype frequencies**

The RR genotype was most prevalent in Jersey cows (0.40), and lowest in crossbred cows (0.26). The rr genotype was found only in crossbred HF cows (0.24). The frequency of R allele was highest in Jersey cows (0.70). The crossbred cows harbour the highest frequency of r allele (0.50). The non-significant (P>0.05) Chi-square value for all the genotypes showed that the population was in Hardy-Weinberg equilibrium (Table 1). Similar results were reported in different breeds in cattle [9, 11, 14, 15]. The results obtained in the present study are not in agreement with those reported in Red Pied cattle [16], in Russian Black Pied and Red Pied cattle [17, 18]

**Association studies**

Genotype RR effects were found to be significant ($p$<0.05) on all yield traits under in Crossbred HF and Jersey cows (Table 2). The effect of genotype was found to be significant ($p$<0.05) on protein%, fat% and density in Crossbred HF cows (Table 3) and non-significant ($p$>0.05) in Jersey cows (Table 3). The results revealed in the present study were in accordance as reported in Gir and Kankrej Cattle [15], Russian Red Pied cattle [16], Russian cattle [17], two Russian cattle breeds [18], Korean dairy cattle [19] and Black-and-White and Jersey cattle [20].

**Table 1:** Genotypic and allelic frequencies in Crossbred and Jersey cows.

| Gene | Genotype | Frequency | Allele | Frequency | Chi-square |
|---|---|---|---|---|---|
| PRL | RR | 0.26 | R | 0.50 | 0.002 |
| Crossbred | Rr | 0.50 | r | 0.50 | |
| | rr | 0.24 | | | |
| | RR | 0.40 | R | 0.70 | 0.002 |
| Jersey | Rr | 0.60 | r | 0.30 | P>0.05; NS |

**Table 2:** Effect of PRL genotypes and breed on milk yield traits

| Breed | Genotype | N | Av. Milk yield (MY) kg | Av. Protein yield(PY) kg | Av. Fat yield (FY) kg |
|---|---|---|---|---|---|
| Crossbred | RR | 17 | 234.13±10.15$^{a}$ | 0.72±0.13$^{a}$ | 0.76±0.66$^{a}$ |
| | Rr | 28 | 155.13±10.18$^{b}$ | 0.65±0.12$^{b}$ | 0.58±1.48$^{b}$ |
| | rr | 12 | 214.11±10.18$^{a}$ | 0.70±0.13$^{a}$ | 0.79±0.20$^{a}$ |
| Jersey | RR | 9 | 178.16±10.11$^{b}$ | 0.51±0.19$^{b}$ | 0.58±0.03$^{b}$ |
| | Rr | 12 | 288.15±10.18$^{a}$ | 0.78±0.12$^{a}$ | 0.82±0.01$^{a}$ |

NS: Non-significant

a, b, c: means with same superscripts are not significantly different ($P$<0.05) from one another.

**Table 3:** Effect of PRL genotypes and breed on milk quality traits

| Parameters | N | Protein (%) | Lactose (%) | Fat (%) | SNF (%) | Density | Ash (%) |
|---|---|---|---|---|---|---|---|
| Xbred HF | | | | | | | |
| RR | 32 | 3.21±0.12 [a] | 4.48±0.01 [NS] | 4.55±0.16 [a] | 8.57±0.19 [NS] | 27.44±1.02 [a] | 0.69±0.00 [NS] |
| Rr | 53 | 3.13±0.17 [b] | 4.41±0.01 | 3.70±0.17 [b] | 8.52±0.18 | 26.79±1.15 [b] | 0.69±0.00 |
| rr | 25 | 3.16±0.16 [b] | 4.45±0.01 | 4.50±0.12 [a] | 8.55±0.17 | 26.98±1.09 [b] | 0.69±0.00 |
| Jersey | | | | | | | |
| RR | 21 | 3.12±0.00 [NS] | 4.36±0.02 [NS] | 5.26±0.11 [NS] | 8.43±0.15 [NS] | 26.08±0.14 [NS] | 0.68±0.01 [NS] |
| Rr | 15 | 3.11±0.01 | 4.36±0.06 | 5.30±0.13 | 8.38±0.19 | 26.04±0.15 | 0.68±0.01 |

NS: Non-significant

a, b, c: means with same superscripts are not significantly different ($P$<0.05) from one another.

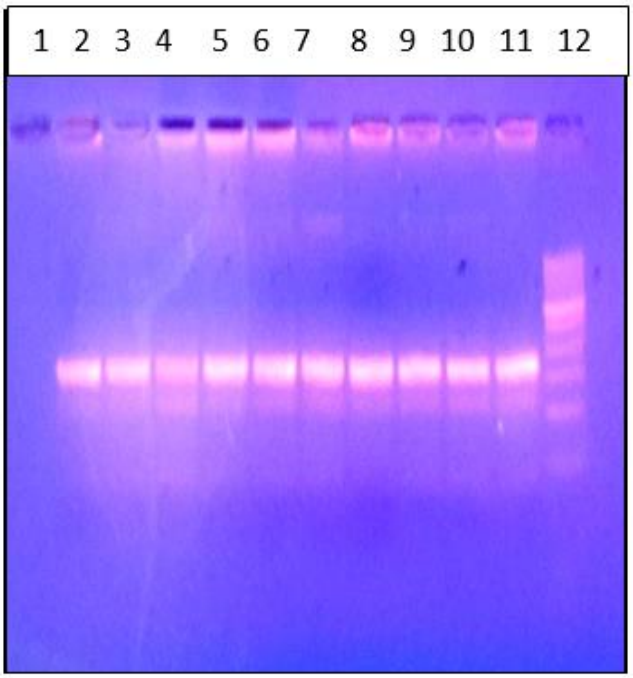


**Plate 1:** Amplification of Exon IV (294 bp) PRL gene Lane 1: Control Lanes 2-6: Jersey cows Lanes7-11: Crossbred HF cows Lane 12: 100 bp ladder

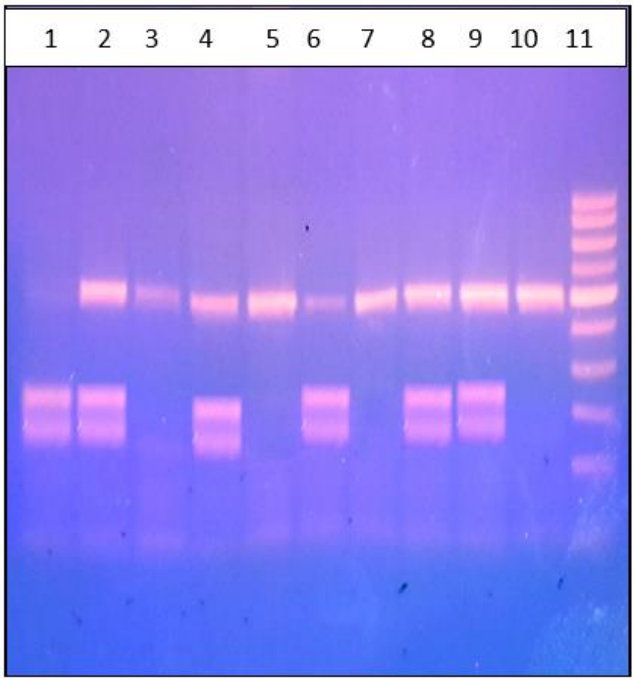


**Plate 2:** Polymorphism of PRL gene with Rsa I enzyme. Single band 294 bp: RR genotype Two bands 162 and 132 bp: rr genotype Three bands 162, 132 and 294 bp: Rr genotype Lanes 1-5: Crossbred HF cows Lanes 6-10: Jersey cows Lane 11: 50 bp DNA ladder

## Conclusion

The populations of Crossbred HF and Jersey cattle were polymorphic and R allele was commonly found. The effect of genotype was found to be significant ($p$<0.05) on quality and yield of milk suggesting that Prolactin gene is associated with high yield and quality of milk.

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