



***Prl* and *B4GALT-1* gene polymorphism and their association with milk production traits in crossbred cattle of Kerala**

TINA SADAN¹, JAMUNA VALSALAN², THIRUPATHY VENKETACHALAPATHY³ and T V ARAVINDAKSHAN⁴

Kerala Veterinary and Animal Sciences University, Thrissur, Kerala 673 576 India

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ABSTRACT

The main objective of present study was to explore the genetic variation in exon 3 of *PRL* (185 bp) and exon-2 of *B4GALT-1* (256 bp) locus and analyze their associations with milk production traits. The study was conducted on 200 crossbred cattle distributed in different farms under Kerala Veterinary and Animal Sciences University and field centres of ICAR-FPT scheme, Mannuthy. Polymorphism of both the genes was detected by Single Strand Confirmation Polymorphism (PCR-SSCP). Similar banding pattern were noticed in exon-3 region of *PRL*, population was found to be monomorphic, indicating conserved nature of this fragment in the screened crossbred cattle population of Kerala. Two unique band patterns were detected in 256 bp fragment of *B4GALT-1*. Sequencing revealed a non-synonymous single nucleotide variation in c.521T>C in exon 2 of *B4GALT-1*, resulted in an amino acid substitution of methionine to threonine due to a codon change of ATG to ACG. Different genetic variants of *B4GALT-1* was significantly associated with 305 days milk yield and protein percent. The study indicates the existence of genetic variability in *B4GALT-1* gene on crossbred cattle population of Kerala and suggests a scope of considering genetic variants of *B4GALT-1* gene in selection of cattle for higher milk production.

Keywords: *B4GALT-1*, Crossbred cattle, Milk yield, Polymorphism, *PRL*

Crossbred cattle play an integral role in the agriculture sector of Kerala. As per Basic Husbandry Statistics (BAHS, 2019), total cattle population of Kerala was 1.33 million; out of which, 1.25 million (94.20%) were crossbred and only 0.08 million (5.80%) were indigenous breeds. In Kerala, about 2.35 million tonnes of milk is produced by crossbred cattle which constitutes 99% of the total milk production of the state. Crossbred cattle population of Kerala have exotic inheritance mainly from Holstein-Friesian (HF), around 50–65%.

High milk yield is an important determinant of performances of cattle which affects overall profitability. Milk production traits are polygenic, affected by many genes and variants, each with small effects on the observed phenotype. Improvements in management and nutrition, along with intense genetic selection have increased milk production and productivity of cattle in recent decades (Vande *et al.* 2016). To augment the selection response for milk yield in dairy cattle, traditional selection methods could be complemented with gene assisted or marker assisted selection (MAS) using genomic variation of the traits (Wakchaure *et al.* 2015). The optimistic candidate genes affecting milk performance traits in dairy cattle should be identified, on the basis of associations between markers or

genetic variations on the gene and the phenotypes under study (Snelling *et al.* 2013, Nayeri *et al.* 2016).

Prolactin gene (*PRL*) is a potential quantitative trait locus and can be used as a genetic marker related to milk performance traits which encodes for a polypeptide hormone called prolactin (Mehmannavaz and Ghorbani 2012). *PRL* is one of the most versatile hormones of the pituitary gland plays crucial roles in mammary gland development, initiation and maintenance of lactation. They are also primarily responsible for the synthesis of milk proteins, lactose, lipids and all other major components of milk (Horseman *et al.* 1997). Multiple studies reported that *PRL* is associated with milk production and its quality (Brymet *et al.* 2005, Ghasemie *et al.* 2009). Therefore, the bovine prolactin gene (located on chromosome 23 in cattle) seems to be a reliable candidate for quantitative trait loci (QTL) affecting milk production traits. He *et al.* (2006) identified G/T SNP in position 485 of prolactin gene promoter in Chinese Holstein cows with three genotypes. Further association analysis showed that cows with genotype BB had significantly higher milk yield, fat yield and protein yield than those of genotype AA and AB.

β(1,4)-galactosyltransferase-I gene (*B4GALT-1*) is an important galactosyltransferase and widely distributed enzyme found in mammals considered as a reasonable candidate gene (located on chromosome 8 in cattle) for milk production in dairy animals (Shahbazkia *et al.* 2010). *B4GALT-1* encodes the catalytic part of the enzyme lactose

Present address: ¹Kerala Veterinary and Animal Sciences University, Thrissur, Kerala. ✉Corresponding author email: jamuna@kvasu.ac.in

synthase responsible for tissue specific production of lactose in the mammary gland. Lactose is the major carbohydrate component and most important osmolyte in milk. It regulates the osmotic pressure and volume of the milk. Milk volume and composition is potentially affected by lactose production (Vilotte 2002). Shahbazkia *et al.* (2012) reported 18 different genotypes and nine polymorphic nucleotide sites in different exons of *B4GALT-1* and also found that milk production traits in Iranian Holstein cattle were significantly associated with different genotypes at exon 2 of *B4GALT-1*.

However, scanty research had been done to explore SNPs within exon 3 of *PRL* gene and exon 2 of bovine *B4GALT-1* gene in Holstein Friesian crossbred cattle. Therefore, the main objectives of present study were to identify the variations at nucleotide level in exon 3 of *PRL* and exon 2 of bovine *B4GALT-1* in the crossbred cattle of Kerala using PCR-SSCP technique and further analyze their associations with milk production traits.

MATERIALS AND METHODS

Animals and sample collection for DNA isolation: Crossbred cattle (200) maintained at the University Livestock Farm, Mannuthy; Cattle Breeding Farm, Thumburmuzhy and different field centres of ICAR Field Progeny Testing Scheme, Mannuthy were used in the present research for the polymorphism analysis. Venous blood (5 mL) was collected from the jugular vein of each animal into vacutainer tubes containing EDTA as anticoagulant and stored at 4°C until processing. Genomic DNA was isolated from blood samples following the phenol-chloroform extraction method described by Sambrook and Russel (2001).

PCR amplification: Two pairs of primers were designed to amplify exon 3 of *PRL* (P_F 5'-GAATGCCCAAACAAC-CCTAA-3', P_R 5'-TCTGGTGAAAACCCGGATAA-3') and exon 2 of *B4GALT-1* (P_F 5'-TTCCCCACCTCTCT-AGTCG-3', P_R 5'-CACCTGGTTGATAACATAGATGC-3') according to its genomic sequence (GenBank Accession No. NC_037350 and NC_037335 whole genome shotgun sequence) using the Primer-3 (V.0.4.0) software. PCR amplification (25 µl, final volume) was performed in thermal cycler (BIORAD, USA) using 50 ng of bovine genomic DNA, 1×PCR buffer, 1.8 mM MgCl₂, 0.2 mM each dNTP, 10pM of each primer and 1U of *Taq* DNA polymerase. Conditions were one cycle at 95°C for 3 min, followed by 35 cycles (30 sec at 94°C, 45 sec at 58.3°C for *PRL* and 63°C for *B4GALT-1* and 45 sec at 72°C), followed by 1 cycle at 72°C for 5 min, stopped at 4°C. Amplified PCR products were loaded into the wells of 2% agarose gel with a standard 50 bp DNA ladder (GenerRuler, MBI Fermentas, Germany) as a marker to check the size of the fragment. Electrophoresis was carried out at the rate 6 V/cm in 1× TBE buffer. Gels were stained with ethidium bromide and visualized under UV light and documented in a gel documentation system (Bio-Rad, USA).

SSCP and SNP identification: Genotyping of samples

was done by Single Stand Confirmation Polymorphism (SSCP) analysis using vertical electrophoresis (BIORAD, USA). The amplified fragments were mixed with SSCP loading buffer in the ratio of 1:2 (6 µl sample with 12 µl dye), denatured at 95°C for 10 min and immediately snap chilled in ice. Products were run in 12% poly-acrylamide gel at 4°C at 120 V, 2 h 30 min for *PRL* and 3 h for *B4GALT-1*. Gels were stained with silver nitrate as per the procedure described by Byun *et al.* (2009) and SSCP fragments were visualised directly. Individual genotypes were defined according to band patterns. Representative PCR products, which showed different banding patterns in SSCP, were sequenced to detect nucleotide variations and aligned with other sequences in GenBank employing BLASTn from NCBI.

Data collection: To evaluate the association of genetic variants with milk production and related performance traits, first lactation data of 305 days milk yield, fat, SNF, lactose and protein percentage were recorded.

Statistical analysis: Popenge32 Version 1.32 was used to estimate the allele and genotype frequencies, observed heterozygosity, expected heterozygosity and Hardy Weinberg Equilibrium. Association analysis of genotypes with milk production traits were analyzed using fixed General Linear Model (GLM) of SPSS V.21.

Fixed model used:

$$Y_{ijklm} = \mu + S_i + P_j + C_k + R_l + G_m + e_{ijklm}$$

where, y_{ijklm} is the milk performance trait measured on the $ijklm^{th}$ animal (305 days milk yield/fat/SNF/lactose/protein percentage); μ is the overall mean of the trait; S_i is the effect of season of calving (winter, summer, rainy, autumn); P_j is the effect of period of calving from 2004 to 2016 (1 to 13); C_k is the effect of centre (1 to 3); R_l is the effect sire (1 to 79); G_m is the effect of genotype (1,2) and e_{ijklm} is the random residual error associated with each observation which is normally and independently distributed with mean zero and unit variance.

RESULTS AND DISCUSSION

PCR-SSCP analysis of *PRL*: SSCP pattern for 185 bp fragment of *PRL* showed similar pattern of two bands (CC) for all samples and the screened population was found to be monomorphic with respect to this fragment. Various exonic loci of Bovine-*PRL* are reported to be highly polymorphic in many cattle breeds. Pawel *et al.* (2005) identified six SNPs within the exon 4 of *PRL* in Polish Black-and-White cows, one of which was shown to be associated with milk yield and fat content. Alfonso *et al.* (2012) three genotypes AA, AB and BB with frequency ranging from 0.776 for BB to 0.026 for BB by PCR-RFLP analysis using *RsaI* enzyme in American Swiss cattle. They also revealed that that animals with genotype AA had a greater milk production during lactation than genotypes AB and BB ($P < 0.05$). A point mutation from adenine-guanine (A103G) was also observed Patel and Chauhan (2017) by PCR-RFLP analysis in exon 3 region of *PRL* of Gir and

Table 1. Diplotype and haplotype frequencies of exon 2 (256 bp fragment) of *B4GALT-1* based on SSCP pattern

Parameter	Total (200)
Diplotype Frequency	
DD	0.83 (165)
BD	0.18 (35)
Haplotype Frequency	
D	0.91 (365)
B	0.09 (35)
χ^2	1.83
P-value	0.18

Figures in parenthesis represent number of observations.

Kankrej Cattle. Present result suggests the conserved nature of this fragment in the screened crossbred cattle population of Kerala.

PCR SSCP analysis of *B4GALT-1*: PCR-SSCP analysis of the 256 bp fragment revealed two different band patterns. Hence the locus is denoted as polymorphic in the screened population with genotypes, viz. DD and BD. Genotype and allelic frequencies are presented in Table 1. On evaluating the population indices, it is observed that DD genotype (0.83) was predominant than BD genotype (0.18). Allele D was most abundant with frequency of 0.91 and allele B was observed at a frequency of 0.09 in the screened population. Chi-square analysis revealed that the screened population is under Hardy Weinberg equilibrium ($\chi^2=1.83<3.841$).

Nucleotide sequencing and SNP detection: On sequencing PCR products from each pattern, one SNP (T→C) transition was revealed at 115th position of 256 bp fragment. The observed SNP was located in nucleotide sequences at positions 97 in exon 2 of *B4GALT-1* and at 521th position of ORF. Further analysis revealed c.521T>C was a non-synonymous mutation and it resulted in an amino acid substitution of methionine to threonine due to a codon change of ATG to ACG. In agreement with present result, Shahbazkia *et al.* (2010) reported a T→C transition in exon 2 of *B4GALT-1* in Iranian Holsteins cattle exhibiting two genotype with their respective frequency of 0.94 and 0.06 and allele frequency as 0.97 and 0.03. They also reported one G → C transversion in exon 2 with codon change of CAG to CAC causing amino acid substitution of Glutamine to Histidine.

Association of *B4GALT-1* genotypes with milk performance traits of crossbred cattle: In the association study of *B4GALT-1* genotypes with milk performance traits, 305 days milk yield was significantly influenced by centre and genotype ($P<0.05$). Crossbred cattle from Cattle Breeding Farm, Thumburmuzhy were having higher milk yield than cattle from other centres. This might be due to the better climate, management and availability of green grass in Thumburmuzhy. DD genotype had higher estimates for 305 days milk yield compared to BD genotype. Least-squares mean of 305 days milk yield for DD and BD genotypes of *B4GALT-1* gene were 2,630.02 kg and

2,544.45 kg, respectively. Protein percent was found to be significantly affected by only genotype ($P<0.05$). Significantly lower protein percent was observed for cattle with genotype DD (2.98%), compared to the heterozygous genotype BD (3.08%). SNF and fat percent was not significantly associated with any of the fixed factors.

Minimal research reports were available on association study of genotypic variants of *B4GALT-1* with milk yield and its constituents in cattle. Shahbazkia *et al.* (2012) reported 18 different genotypes and nine polymorphic nucleotide sites in different exons of *B4GALT-1* and also found that milk production traits in Iranian Holstein cattle were significantly associated with different genotypes at exon 2 of *B4GALT-1*. Overview of results suggested that genetic variants of *B4GALT-1* can be used as a marker for selecting crossbred cattle of Kerala for higher milk yield and its constituents mainly protein.

PCR amplification of exon-3 of *PRL* and exon-2 of *B4GALT-1* yielded an amplified product of 185 bp and 256 bp respectively. PCR-SSCP analysis of *B4GALT-1* revealed two types of genotypes DD and BD in crossbred cattle of Kerala with a frequency of 0.83 and 0.18 respectively. Sequencing revealed a non-synonymous single nucleotide variation in c.521T>C in exon 2 of *B4GALT-1*, resulted in an amino acid substitution of methionine to threonine due to a codon change of ATG to ACG. Different genetic variants of *B4GALT-1* in crossbred cattle is significantly associated with 305 days milk yield and protein percent. Overall study indicates the existence of genetic variability in *B4GALT-1* gene on crossbred cattle population of Kerala and suggests a scope of considering genetic variants of *B4GALT-1* gene in selection of cattle for higher milk production.

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