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Association of Butyrophilin gene polymorphism (A465G) with milk production traits in Holstein Friesian crossbred cattle of Kerala

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Abstract

The butyrophilin (BTN1A1) gene is found at a quantitative trait locus (QTL) in cattle for milk production traits. The effect of a single nucleotide polymorphism (SNP) A465G in exon 8, which causes a lysine to arginine transition, on milk production attributes in Holstein Friesian crossbred cattle from Kerala was investigated in this study (151 numbers). Using custom synthesised primers, a 90-bp fragment encompassing the polymorphic region was amplified from genomic DNA isolated. Genotyping was carried out by high resolution melt curve analysis (HRM) and two genotypes KK (0.25) and KL (0.75) were detected based on melting temperature and melt curve patterns. Sanger's sequencing and sequence analysis of representative samples confirmed the genotypes. Chi-square test showed that the population was not distributed as per Hardy-Weinberg equilibrium (p≤0.05). The relationship between the A465G transition and milk production traits like 305 day milk, fat, SNF yields, fat and SNF per cent was determined by general linear model-analysis of variance (GLM-ANOVA). In the model herd, season of calving, parity of animal (non-genetic factors), and genotype were considered fixed variables and milk production traits as dependent variable. The study revealed significantly higher (p≤0.01) milk fat and SNF per cent for KK genotype (4.14±0.08 %; 7.86±0.06 %) than KL genotype (3.91±0.07 %; 7.73±0.05 %). The butyrophilin gene polymorphism (A465G) can be recommended as a marker for higher milk fat and SNF per cent in future breeding programmes in crossbred cattle of Kerala.

Keywords: *Butyrophilin, SNP, high resolution melt curve, crossbred cattle, milk production traits*

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Butyrophilin (*BTN1A1*) gene is mapped to bovine autosome 23 (Taylor *et al*., 1996) and in the same region quantitative trait loci (QTL) for milk production traits (Ashwell *et al.*, 1996; Bennewitz *et al.*, 2004). Previous researchers suggested *BTN1A1* as a candidate gene for milk production (Komisarek *et al.*, 2006; Wenjun *et al*., 2009; Muszynska *et al*., 2010; Al-Waith 2019) and disease resistance traits (Smith *et al*., 2010) in cattle. Butyrophilin gene is present on juxta-telomeric region of bovine leucocytic antigen (BoLA) complex (Ashwell *et al*., 1996) and span over 7003 bp length with eight exons and seven introns (Vishwanath, 2014). Butyrophilins are type I trans membrane proteins belonging to immunoglobulin (Ig) super family with an extra cellular andcytoplasmic domain. Cytoplasmic domain of *BTN1A1* tightly binds to xanthine dehydrogenase (*XDH*) to form a supra-molecular complex. This complex binds to adipophilin in the phospholipid bilayer of milk secretory granule and pinches off the mammary gland epithelial cell in zipper like fashion (Robenek *et al*., 2006).

Many molecular markers in candidate genes for milk production such as *Diacylglycerol-o-transferase 1* (Lali and Anilkumar, 2016), *Beta 1,4-galactosyltransferase-1* (Valsalan *et al.,*2021), *Leptin* (Lali and Bindu, 2015) and *Osteopontin* (Lali *et al.,* 2020) were studied in crossbred cattle of Kerala. It is worth to note that, majority of SNPs act as indirect markers and the influence on production traits depends on state of linkage disequilibrium (LD) in cattle. Schmid and Bennewitz (2017) explained that the LD between the marker and the QTL might be affected by the genetic recombination during gamete formation. It reiterates the importance of association results of local populations to confirm the effects of markers especially indirect markers. The influence of *BTN1A1*gene polymorphisms was not studied yet in the crossbred cattle population of Kerala. The present study was designed to analyse the effect of polymorphism in*BTN1A1* gene (A465G) on milk production traits in Holstein Friesian crossbred cattle of Kerala.

Materials and methods

Estimation of milk production traits

Milk samples were collected from 151 crossbred cattle maintained at University Livestock Farm and Fodder Research Development Scheme (ULF-FRDS), Mannuthy and cattle breeding farm (CBF), Thumburmuzhy once in a month for ten months. The samples were brought to laboratory under refrigerated conditions. Data regarding animal number, date of calving, parity, test day milk yield, recording month and date were obtained from farm records. Test day fat per cent was estimated from automatic milk analyser (MRC instruments) only after routine standardisation with Gerber's centrifugation method. Data regarding test day SNF per cent was derived from milk analyser by running samples. Test interval method (TIM), according to guidelines of international committee for animal recording (ICAR, 2020) was used to calculate 305 day yields of milk, fat and SNF along with 305 day milk fat and SNF per cent.

High resolution melt curve analysis

A volume of 5 mL blood was collected from jugular vein of 151 crossbred cattle in a EDTA coated vial. Genomic DNA was isolated from blood by standard phenol chloroform extraction method (Sambrook and Russell, 2001). Isolated DNA was checked for concentration and purity by Nanodrop spectrophotometry. Quality was assessed by 0.8 per cent agarose gel electrophoresis.

Polymorphism analysis and genotyping were carried out using High resolution melt curve (HRM) analysis (Desai *et al.*, 2021) in Eco Real-Time PCR system (Illumina). Primers were custom synthesised using primer3 V.0.4.0 software and a gradient PCR was carried out to fix optimum annealing temperature and specific amplification was detected by running samples on 2.5 per cent agarose gel using molecular weight marker of 50 bp size. The HRM reaction was carried out using SSO FAST EVA GREEN super mix, forward (**5**´GCCCTTCTTCTGCTTGTGGT**3**´) and reverse (**5**´TCAGCAACTACCATGACTCCC**3**´) primers and template DNA. Thermal profile of reactions include 94°C for 5 min, 94°C for 30s, 62.5°C for 30s, 72°C for 30s followed by melt curve analysis. The results were confirmed

by Sanger's sequencing the representative samples from each genotype after detecting them using melting temperature (T_m) shift and melt curve pattern. Further, the genotype and allele frequencies were calculated and population was checked for Hardy Weinberg equilibrium using *chi*-square test.

Association with milk production traits

In order to study the association of A465G polymorphism with milk production traits, General linear model-Analysis of variance (GLM-ANOVA) was performed using SPSS version 24.0. Non-genetic factors such as herd, season of calving (October to January – post monsoon, February to May – summer, June to September – monsoon) and parity (1 to 4) and milk production traits 305-day milk yield, fat yield, fat per cent, SNF yield and SNF per cent as dependent variables.

The model was $Y_{ijklm}= \mu+H_i+S_j+P_K+G_l+e_{ijklm}$

Where,Y_{ijklm} – trait of m^{th} cow in i^{th} herd, j^{th} season, k^{th} parity and belonging to l^{th} genotype, μ - population mean of trait, H_i – effect of i^{th} herd (i = 1 or 2), S_j – effect of j^{th} season (j = 1 to 3), P_k – effect of k^{th} parity (k = 1 to 4), G_l – effect of l^{th} genotype (l = 1 or 2) and e_{ijklm} – Random error.

Results and discussion

A single nucleotide polymorphism in the exon 8 of *BTN1A1*gene resulting from adenine to guanine transition was studied in detail in the present study by high resolution melt curve analysis in HF crossbred cattle of Kerala.

High resolution melt curve analysis

Gradient PCR detected optimum annealing temperature of 62.5 °C. Specific amplification was confirmed by running amplicons through 2.5 per cent agarose gel (Fig. 1). Melt curve analysis clearly depicts presence of two curve patterns representing two genotypes KK and KL (Fig. 3) in the studied population. According to classification of Venter *et al.* (2001), the typical melting temperature shift of C/T or G/A mutations should be >0.5°C. Similarly, the genotypes of HRM analysis of the current study showed a melting temperature shift around 0.6°C. The chromatograms of both genotypes are depicted in Fig. 3. The sequence results were checked for any other genetic variations in the population since, multiple variations in the same amplicon will interfere the interpretation of HRM results.

The allele K (0.63 %) and genotype KL (0.75 %) were found to be frequent in the studied population (Table 1). The frequency of dominant allele K in different studies were0.88 by Komisarek and Dorynek (2003), 0.83 by Bhattacharya *et al*. (2006), 0.86 by Sadr *et al*. (2008), 0.88 by Rengarajan (2011), 0.89 by Vishwanath (2014) and 0.86 by Al-Waith (2019). Thus, the results of K

Table 1. Genotype and allele frequencies for SNP A465G in bovine *BTN1A1* gene

SNP	Genotype frequencies			Allele frequencies		*Chi*-square value
A465G (n=151)	KK/0.25 (37)	KL/0.75 (114)	LL/0 (0)	K/0.63	L/0.37	55.54^S

S-Significant (p≤0.05)

Table 2. Effect of SNP A465G on milk production traits in crossbred cattle of Kerala

Sl. No.	Trait (Mean ± SE)	A465G		p- value
		KK	KL	
1.	305 day milk yield (kg)	2764.35±157.96	2850.68±128.20	p>0.05
2.	Fat yield (kg)	112.40±5.50	109.73±4.52	p>0.05
3.	Fat per cent	4.14±0.08	3.91±0.07	p≤0.01
4.	SNF yield (kg)	216.05±11.75	219.70±9.54	p>0.05
5.	SNF per cent	7.86±0.06	7.73±0.05	p≤0.01

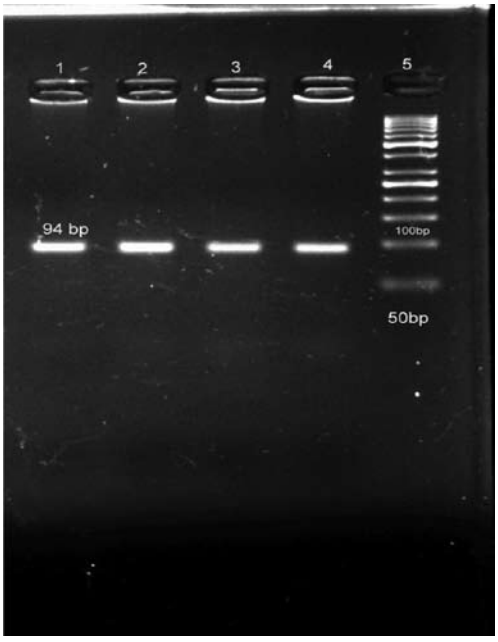

Fig.1. *BTN1A1* gene fragment (94 bp) on 2.5 % agarose gel
Lane 1-4: 94 bp product enclosing exon 3
Lane 5 : 50 bp ladder

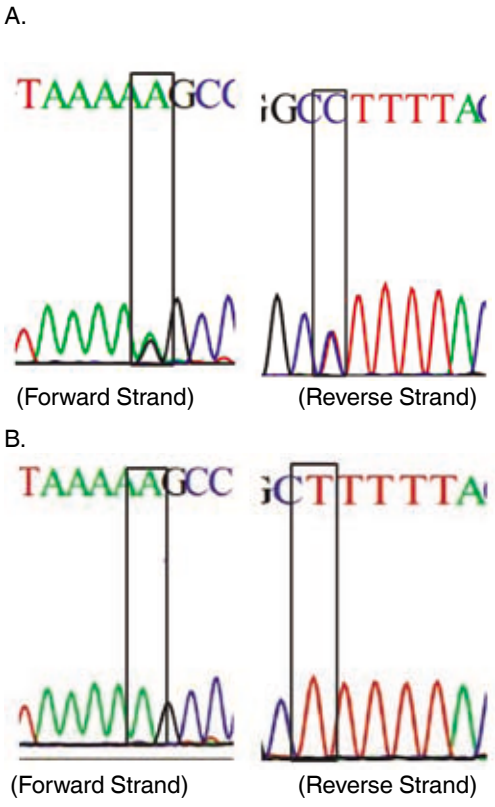

Fig. 2. Chromatogram depicting SNP A465G in exon eight of *BTN1A1* gene in crossbred cattle of Kerala. A and B – Chromatogram depicting KL and KK genotypes, respectively

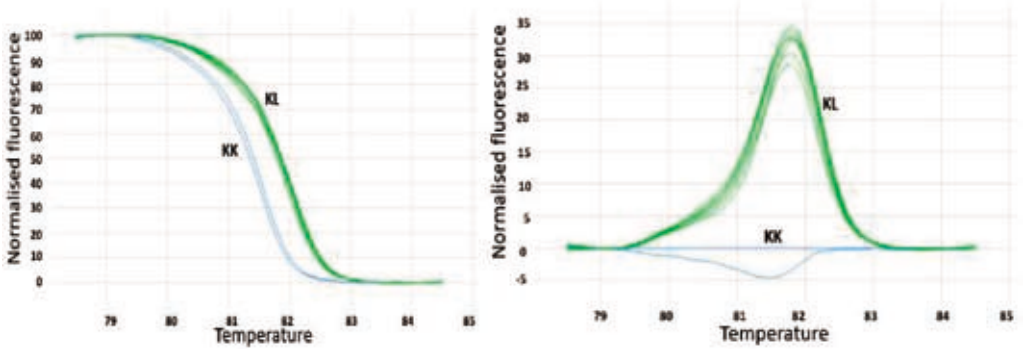

Fig. 3. Normalised and difference melt curve of polymorphism A465G representing two different curve patterns for genotypes KK (blue) and KL (green), respectively.

allele dominance was in accordance with all aforementioned studies. The genotype LL was not detected in the present study which was in agreement with Sadr *et al*. (2008), Rychtarova *et al*. (2014), Vishwanath (2014) and Al-Waith (2019). *Chi*-square test showed that the population was not in Hardy-Weinberg equilibrium ($p \leq 0.05$). Natural selection may be going on favouring the K allele in crossbred cattle population. However, the status of HW equilibrium has to be further examined in a larger population.

Association with milk production traits

The two genotypes KK and KL of A465G transition of HF crossbreds of Kerala differed significantly ($p \leq 0.01$) with respect to fat and SNF per cent (Table 2) as per GLM ANOVA considering herd, season of calving, parity of animal (non-genetic factors) and genotype as fixed variables and milk production traits as dependent variables. Similar results were obtained by Rengarajan (2011) in milk fat per cent and current findings were also in accordance with Vishwanath (2014). However, contradictory to the present results, animals with KK genotype in a study by Komisarek *et al*. (2006) showed significant higher yields of milk, fat and SNF. Furthermore, Rychtarova *et al*. (2014) detected no association of the SNP A465G with milk production traits and it agrees with the current results except for fat and SNF per cent. Al-Waith (2019) identified significant difference between two genotypes KK and KL with respect to milk yield (KK had higher milk yield) and fat per cent (KL had higher milk fat per cent). Inconsistent association analysis results could be the result of population substructure creation, null alleles in population or excessive selection pressure (Lali *et al*., 2020).Thus, A465G polymorphism can be suggested as a potential marker for obtaining a good selling price to a farmer for milk as the fat and SNF per cent in milk determines the milk pricing in Kerala.

Conclusion

The study designed HRM analysis to genotype A465G transition in exon eight of bovine *BTN1A1* gene. Genotype KK had significantly higher fat and SNF per cent in HF crossbreds of Kerala. The favourable allele K was found to be frequent in the population and it indicates that selection undergoes towards the favourable genotypes. Presently more emphasis is on milk composition traits and so extensive studies such as genome wide association studies are required to find out the genes regulating the milk components in cattle. Along with these, studies directing to find influence of stage of lactation are also necessary as it was found that this particular factor has effect on milk composition traits (Prasad and Subramanyam, 1986).

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Conflict of interest

The authors report no conflict of interest.

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