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Novel polymorphisms of the *KCNB1* gene and their association with production traits in Indian Sahiwal cattle

RAGINI KUMARI[1], RAKESH KUMAR[2]✉, BEENA SINHA[3], REBEKA SINHA[4], ISHWAR DAYAL GUPTA[5] and ARCHANA VERMA[5]

ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

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ABSTRACT

Mastitis in cattle is a prevalent mammary gland disease that contributes significantly to the increase in veterinary expenditures in the dairy sector. *KCNB1* (Potassium voltage-gated channel, subfamily B member 1) gene is involved in regulating apoptosis, cell proliferation and differentiation, udder epithelial tissue maintenance and repair, mammary gland development and recommended as a candidate gene for production related traits in cattle. The purpose of this research was to detect the genetic variants of *KCNB1* gene in Sahiwal cattle and to analyze the association between polymorphisms with milk production traits, udder traits, and teat traits in Sahiwal cattle. A total of 87 cattle were genotyped by polymerase chain reaction-restriction fragment length polymorphism technique. Two single nucleotide polymorphisms within the non-coding sequence of *KCNB1* gene were identified (g.78216220G>A and g.78216335A>G). Analysis of productivity traits within the genotyped animals revealed that the SNP1-Msp1 locus (g.78216220G>A) located at intron 1 was associated with milk production traits, but the SNP2-BspHI locus (g.78216335A>G) had no association with milk production. Significant associations were also observed between SNP1-Msp1 and SNP2-BspHI loci with both udder and teat traits. Our results demonstrate that polymorphisms in the cattle *KCNB1* gene were associated with milk production, udder and teat traits and might be utilized as a genetic marker for marker-assisted selection in cattle breeding programs.

Keywords: Cattle, *KCNB1*, Mastitis, Polymorphism, Production, Sahiwal

Mastitis, also known as inflammation of the mammary gland, is now recognised as one of the diseases that place the greatest financial burden on dairy producers all over the globe (Nash *et al.* 2000). In the dairy sector, yearly losses of almost 2 billion $ occur due to mastitis in the USA and 526 million $ in India, whither subclinical mastitis is responsible for about 70% of the total economic losses (Varshney and Naresh 2004). The incidence of clinical mastitis in zebu (Indigenous) breeds had been proclaimed to be 26.4% in Sahiwal cattle (Khate and Yadav 2010). Hitherto, mastitis control programs should be executed to impede mastitis in dairy farms by way of absolute hygiene and management practices (NMC 2009). Numerous studies reported superior genetic gain for overall economic value when selection for mastitis resistance was included in breeding programs than the selection for production trait only (Rogers 1993, Colleau and Bihan-Duval 1995). Genetic selection is the most important criteria that has been used to prevent mastitis in dairy cattle either directly or indirectly (De Haas *et al.* 2002, Degard *et al.* 2002). The direct selection methods for the curb of clinical mastitis in individual animals are not accomplishable, because of very low heritability (0.02 to 0.05), so it is hard to quantify (Mrode and Swanson 1996, Rupp and Boichard 1999). The indirect method of selection of the udder and teat conformation traits are highly heritable and have a relation with milk somatic cell count (SCC), so it plays an important role in the dairy cattle breeding program (Klein *et al*. 2005, Sharma *et al.* 2011). Therefore, in dairy cattle, udder type traits viz. udder attachment, udder depth, udder height, and udder width were taken for genetic selection which makes them capable to enhance milk production efficiency and reducing the chance of mastitis (ICAR 2012). As a result, enhancing breeding programs with genetic traits to improve mastitis resistance and thus avoiding infection might be an effective tool for the dairy sector (Meredith *et al.* 2013). The cattle with elongated teats are more vulnerable to mastitis infection than the inverted teat ends (Seykora 1985). In addition, dairy cattle having traits like broad udders, lower hind-quarters, and teats placed widely assist to invade of the infectious agent and should be selected counter of these traits (Thomas 1984). So, improvement in these traits might improve the herd life and the milk production level

Present address: [1]Chhapra, Saran, Bihar. [2]ICAR-Research Complex for Eastern Region, Patna, Bihar. [3]Kusherhi, Sheikhpura, Bihar. [4]College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. [5]ICAR-National Dairy Research Institute, Karnal, Haryana. ✉Corresponding author's e-mail: rakesh05vet@gmail.com

of dairy cattle (Atkins and Shannon 2002). The majority of Quantitative trait loci (QTL) for udder conformation traits were detected in non-coding regions (Intron) of the genome, which demonstrates that nucleotide variants in regulatory sequences are the prime determinants of disparity in udder morphology in cattle (Pausch *et al.* 2016).

KCNB1 (Potassium voltage-gated channel, subfamily B member 1), a protein coding gene, plays an important role in the management of mastitis. It helps in the regulation of apoptosis, cell growth and differentiation, maintenance and repair of udder epithelial tissue, and development of mammary glands in dairy cattle (Kim *et al.* 2014, Pal 2018). The *KCNB1* gene has been mapped on *Bos taurus* autosome 13 (BTA-13) and spans nearly 110.35 kb and comprising of 2 exons and 1 intron, having a translation length of 858 residues. In Sahiwal cattle, there is currently no information available on the association of genetic variants of the *KCNB1* gene with udder type traits, the incidence of clinical mastitis, and milk production. Therefore, to improve the understanding of the *KCNB1* gene, present study was undertaken to explore the novel nucleotide variations of the Sahiwal cattle *KCNB1* gene and analyzed their associations with udder type and milk production traits. Hence, the SNPs that were revealed in this study have the potential to be used in breeding programs to enhance milk production in dairy cattle.

MATERIALS AND METHODS

Geographical location and climatic description: The animals for conducting research were selected randomly from Livestock Research Complex, National Dairy Research Institute (NDRI), Karnal, Haryana, India. The research farm is located at 29.43°N and 77.20°E coordinates with an altitude of 250 meters above the mean sea level. The region is situated in sub-tropical climate and temperature rises in the summer season (April to June), ranging between 26°C to 45°C, and experiences modest rainfall from July to September with annual precipitation of 700 mm. Whereas, winter season (October to January), are very cold with temperature ranging from 3°C to 33°C.

Ethical approval: All animals were treated in the experiment and plan of study under the strict compliance of the Institutional Animal Ethics Committee (IAEC) of ICAR-National Dairy Research Institute (NDRI), Karnal, Haryana, India.

Experimental animals and data collection: The experimental study was conducted in Sahiwal cattle (*n*=87) maintained at Livestock Research Centre (LRC) of ICAR-NDRI, Karnal, Haryana. The data for udder type traits, milk yield, and incidence of clinical mastitis was recorded for all the animals taken in this study. The milk production parameters viz. 305 days milk yield (kg), total milk yield (TMY) (kg), monthly milk yield (MMY) (kg), and test day milk yield (TDMY) (kg) were collected from the daily milk yield register kept in the livestock record unit and incidence of mastitis was taken from Animal health complex of ICAR-NDRI, Karnal. All the udder conformation traits were taken from the recommendation made by different agencies (ICAR 2012, WHFF 2014, WCGALP 2014, HAU 2016), and a few others were taken according to the importance of traits with milk production and mastitis. The range for each trait was calculated by subtracting the minimum value from the maximum value. As the number of classes in which the animals were evaluated is 9, this range was divided by 9 to get the unit score point. This unit score point was added to that of minimum value to get the range for score 1. The subsequent score classes were obtained by adding a unit score point to the highest unit of previous classes.

Genomic DNA extraction: Blood samples (8-10 ml) were collected aseptically from the Jugular vein of Sahiwal cattle (*n*=87) into a sterile vacutainer tube containing EDTA as an anticoagulant. DNA was extracted from the blood cells by using the phenol-chloroform extraction technique (Sambrook and Russell 2001), diluted to working concentration (50 ng/µL) and preserved at -20°C for use as PCR templates.

PCR amplification and sequencing: In silico primer, designing was carried out using Primer3 software (http://www.primer3.ut.ee) (Untergasser *et al.* 2012) for the *Bos taurus KCNB*1 gene (Ensembl Ref Seq: ENSBTAG00000027320). The primers (F-TTCAAATCCCGACTCCACCA and R-TAACACACAAAAGTCGCCCC) utilized in this study were designed to target a span of 505 bp of bovine *KCNB*1. To genotype, every individual, PCR-restriction fragment length polymorphism was employed. The PCR amplification was performed in a total volume of 25 µL, including 1.0 µL genomic DNA (50 ng/µl), 0.5 µL of every primer (10 pm/µL), 13.0 µL of Fermentas 2× PCR master mix, and added sterile ultrapure water as the final dilution. The thermal conditions of the PCR were initial denaturation for 3 min at 94°C followed by 34 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 62.2°C, elongation for 1 min at 72°C, and a final elongation for 7 min at 72°C. Following that, each PCR product was identified using an agarose gel in 0.5×TBE (tris–borate–EDTA solution) combined with ethidium bromide stain on a 1.5% (Fig. 1) and 3.5% (Fig. 2) agarose gel. Furthermore, the selected amplified PCR products were sent to 1st base sequencing INT (Singapore) for purification and custom sequencing from both ends (5′ and 3′ ends). Sequences were analyzed with the chromas software (version 2.6.6), and each edited sequence was aligned with the corresponding reference sequence (ENSBTAG00000027320) using ClustalW

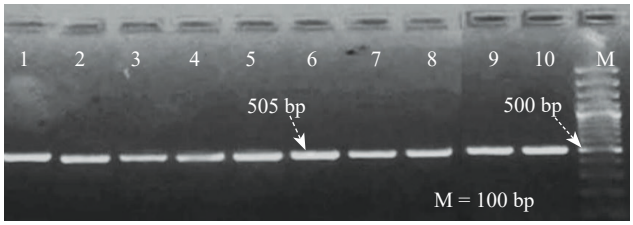


Fig. 1. PCR amplification product of the *KCNB*1 gene in Sahiwal cattle.

multiple sequence alignment programs (www.ebi.ac.uk/tools/msa/clustalw) to identify SNPs.

Statistical analyses: The population parameters viz. allelic frequencies, genotypic frequencies, effective allele number (ne), Shannon Index (I), expected heterozygosity (Nei), and the polymorphism information content (PIC) were estimated by PopGene version 1.32 (Yeh *et al.* 1999). The SHEsis program (http://analysis.bio-x.cn/myAnalysis.php) was used to examine the Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) structure of the two SNPs loci in the studied population (Shi and He 2005).

Associations between SNPs loci with production traits (viz. 305 milk yield, total milk yield, monthly milk yield, and test day milk yield) and udder type traits viz. fore udder attachment (FUA), rear udder width (RUW), rear udder height (RUH), udder balance (UB), udder depth (UD), udder length (UL), udder width (UW), udder circumference (UC), central ligament (CL), teat circumference (TC), fore teat length (FTL), rear teat length (RTL), the distance between fore and rear teat (DFR), distance between the left and right teat (DLR), shortest distance of floor from fore teat (SDF), shortest distance of floor from rear teat (SDR), and teat diameter (TD) was analyzed using a general linear model (GLM) procedure of SPSS Version 22 by the following model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

where, Y_{ij} is the adjusted value of the j^{th} animal's type traits to the i^{th} genotype, μ is the overall mean value, G_i is the fixed effect of i^{th} genotype, e_{ij} is the random error associated with Y_{ij} observation.

In addition, the association between SNPs with the visual traits viz. udder shape (US), udder suspension (US), teat shape (TS), teat end shape (TES), teat size (TS), rear teat placement (RTP), fore teat placement (FTP), skin condition (SC), and long term changes in teat end condition (LTCTEC) were analyzed using chi-square ($\chi 2$) test (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

Detection of Novel SNPs of KCNB1 and genotyping: Sequencing of *KCNB1* within Sahiwal cattle revealed two SNPs, namely, SNP1-2 (Fig. 3). The SNP1-Msp1 locus (g.78216220G>A; Fig. 3a) was located at intron 1 and mutated from G to A, after digestion by the *Msp*1, which generated fragments with lengths of 225 and 280 bp for genotype GG; 505 bp for genotype AA; and 505, 225, and 280 bp for genotypes GA, respectively (Fig. 2a). The SNP2-BspHI locus (g.78216335A>G; Fig. 3b) was too detected in intron 1 and mutated from A to G, digestion with *BspHI*, which generated fragments with lengths of 396 and 109 bp for genotype AA; and 505, 396 and 109 bp for genotypes AG, respectively (Fig. 2b).

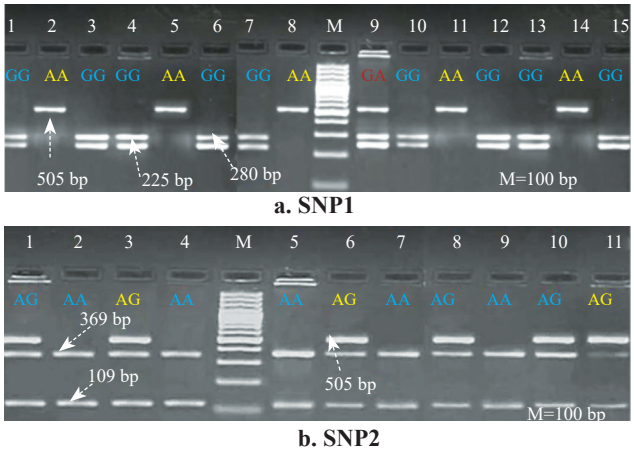


Fig. 2. Electrophoresis pattern of two novel polymorphisms of the KCNB1 gene. a to b represented the electrophoresis pattern of the SNP1-2 loci, respectively (KCNB1, Potassium voltage-gated channel, subfamily B member 1; SNPs, single nucleotide polymorphisms)

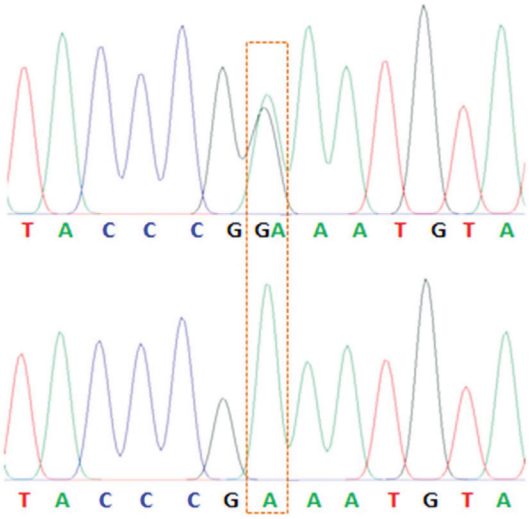
Genetic parameter of KCNB1 gene polymorphisms: Statistical analyses showed that the frequencies of genotypes and alleles are different at different SNPs in Sahiwal cattle (Table 1). Only two genotypes of SNP2-*BspH*I locus, and three genotypes for SNP1-*Msp*1 locus were found in Sahiwal cattle. Consequently, the population indices of various SNPs, including Ho, He, Ne, and PIC, were assessed based on genotypic frequency numbers (Table 1). The PIC values categorization revealed that all SNPs loci, namely, SNP1-*Msp*1 (g.78216220G>A) and SNP2-*BspH*I (g.78216335A>G), had values of 0.364 and 0.307, respectively, indicating medium genetic diversity. The *KCNB1* gene in the Sahiwal cattle population was observed to be away from Hardy Weinberg equilibrium and heterozygote deficiency was also observed.

Linkage disequilibrium analysis of the SNP1-Msp1 (g.78216220G>A) and SNP2-BspHI (g.78216335A>G) loci: The LD of two SNPs loci were analyzed in a studied population. As shown in Fig. 4, the D' and r^2 values of studied population were very low (D'<0.5; r^2<0.2). These values showed that the SNP1 and SNP2 were incompletely

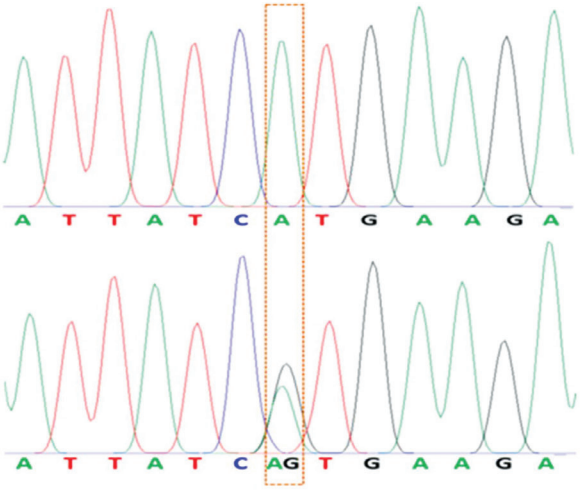
Table 1. Genetic parameters of the *KCNB1* gene in Sahiwal cattle

SNPs	Genotypes	Genotypic frequency	Allele	Allelic frequency	Ne*	I*	PIC*	HWE* test
SNP1-*Msp*1	GG	0.23	G	0.40	1.955	0.681	0.364	Disequilibrium
g.78216220G>A	GA	0.33	A	0.60				
	AA	0.44						
SNP2- *BspH*I	AA	0.51	A	0.75	1.600	0.526	0.307	
g.78216335A>G	AG	0.49	G	0.25				

Ne*, Number of effective alleles; I*, Shannon's index of information; Nei*, Expected heterozygosity; PIC*, Polymorphism information content; HWE*, Hardy–Weinberg equilibrium.



a. SNP1: ENSBTAG00000027320 (g.782116220G>A)



b. SNP2: ENSBTAG00000027320 g.78216335A>G)

Fig.3. Sequence chromas of two novel polymorphisms of the KCNB1 gene. a to b represented the sequence chromas of ENSBTAG00000027320: g.78216220G>A (SNP1-Msp1)and ENSBTAG00000027320:g.78216335A>G (SNP2-BspHI). (KCNB1, Potassium voltage-gated channel, subfamily B member 1; SNPs, single nucleotide polymorphisms)

linked; hence haplotype analysis was not performed.

Association of KCNB1 genetic variants with production, udder, and teat type traits KCNB1 SNPs and production traits: The associations of the genetic variations with production traits i.e. 305MY (kg), TMY (kg), MMY (kg), and TDMY (kg) are given in Table 2. In the SNP1-*Msp*1 locus, the genotype GG (9.81±0.91) had demonstrated

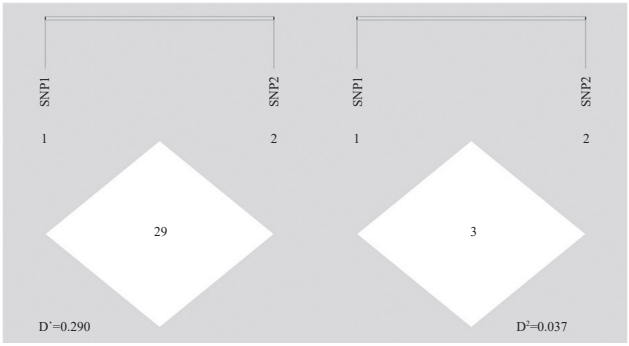


Fig.4. Linkage disequilibrium analyses of the SNP1-Msp1(g.78216220G>A) and SNP2-BspHI(g.78216335A>G) loci of the KCNB1 gene in Sahiwal cattle (D′, 0.290; r^2, 0.037)

significantly (P<0.05) superior test day milk yield (TDMY) compared to AA (7.46±0.66), and GA (7.55±0.75) genotypes, while other production traits were not found significantly associated with any genotypes in studied population (Table 2). Analysis of genotypes within the locus SNP2-*BspH*I revealed that AG genotype produced higher milk yield when compared to those observed in the AA genotype; however, these differences were not statistically significant.

KCNB1 SNPs and udder traits: The different genotypes of SNP1-*Msp*1 locus had significant (*P*<0.05) association with udder circumference (UC), which demonstrated that the genotype GG (148.07±2.35) was superior to AA (139.57±1.70), and GA (141.55±1.95) genotypes, while other udder traits were not found significantly associated with any genotypes (Table 3). For the locus SNP2-*BspH*I, the genotype AA (20.06±0.84) demonstrated superior rear udder height (RUH) and rear udder width (RUW) when compared with the AG genotype (25.41±0.85) in studied population (Table 3).

KCNB1 SNPs and teat traits: The associations of the genetic variations with teat type traits are given in Table 4. The different genotypes of SNP1-*Msp*1 locus had significant (*P*<0.05) association with the distance between fore and rear teat (DFR), which demonstrated that the genotype GA (7.82±0.93) was superior compared to AA (5.46±0.81) and GG (4.76±1.12) genotypes, while genotype GG (28.25±1.10) had significantly superior teat diameter (TD) compared with AA (25.83±0.79), and AG (24.91±0.91) genotypes in studied population. For the locus, SNP2-*BspH*I, it was found that the AA genotype had higher values for all types of teat traits except rear teat length (RTL) and distance between fore and rear teat

Table 2. Associations between the novel polymorphism of the *KCNB1* gene with production traits in Sahiwal cattle

SNPs	Genotypes	305MY (kg)	TMY(kg)	MMY (kg)	TDMY (kg)
SNP1-*Msp*1	GG (20)	2227.21±214.70	2210.87±262.98	247.75±29.55	9.81±0.91[b]
(g.78216220G>A)	GA (29)	1985.54±178.30	1935.37±218.39	183.00±24.54	7.55±0.75[a]
	AA (38)	1708.16±155.76	1592.53±190.79	197.14±21.43	7.46±0.66[a]
SNP2- *BspH*I	AA (44)	1913.63±147.30	1848.81±180.26	194.68±20.10	8.00±0.63
(g.78216335A>G)	AG (43)	1926.40±149.01	1849.11±182.34	213.66±20.34	8.06±0.63

Number of animals is indicated in parenthesis; values with distinct superscripts differ significantly.

Table 3. Associations between the novel polymorphism of the *KCNB1* gene and udder type traits in Sahiwal cattle

SNPs		SNP1-*Msp*1 (g.78216220G>A)			SNP2- *BspH*I (g.78216335A>G)	
Genotypes		GG (20)	GA (29)	AA (38)	AA (44)	AG (43)
Udder traits	FUA (degree)	108.00±4.35	108.17±3.62	105.81±3.16	105.84±2.92	108.40±2.95
	RUH (cm)	21.78±1.36	21.95±1.13	23.34±0.99	20.06±0.84[a]	25.03±0.85[b]
	RUW (cm)	22.15±1.37	22.28±1.14	23.64±1.00	20.34±0.84[a]	25.41±0.85[b]
	UD (cm)	47.65±1.22	48.89±1.01	49.10±0.88	48.36±0.82	49.05±0.83
	UB (cm)	-2.23±0.66	-2.14±0.55	-2.35±0.48	-2.14±0.44	-2.37±0.45
	UL (cm)	51.94±1.38	50.73±1.15	50.30±1.00	51.50±0.92	50.12±0.93
	UW (cm)	53.49±1.40	52.14±1.17	51.59±1.02	52.89±0.94	51.52±0.95
	UC (cm)	148.07±2.35[b]	141.55±1.95[ab]	139.57±1.70[a]	142.34±1.65	142.02±1.67
	CL (cm)	3.41±0.27	3.64±0.22	3.58±0.19	3.60±0.18	3.53±0.18

FUA, Fore udder attachment; RUW, Rear udder width; UD, Udder depth; UB, Udder balance; UL, Udder length; UW, Udder width; UC, Udder circumference; CL, Central ligament. Number of animals is indicated in parenthesis; values with distinct superscripts differ significantly.

(DFR) when compared with the AG genotype; although these differences were not significant.

In the present study, we explored the genetic variations of two novel polymorphisms i.e., SNP1-*Msp*1 (g.78216220G>A) and SNP2-*BspH*I (g.78216335A>G) in intron 1 of *KCNB1* gene and defined their association with mastitis, production, udder, and teat type traits in Sahiwal cattle. The evidence was provided constantly to prove that intron played an important role in regulating post-transcriptional regulation, mRNA splicing, and gene expression although intron did not code protein (Dwyer *et al.* 2021). Therefore, the SNPs that are found in the intron may be crucial for bringing the function of the protein into full play. The indicators of measuring the genetic parameter in population were Ho, He, Ne, and PIC; the superior values of PIC and He imply higher degrees of genetic diversity. It was intermediate polymorphism (0.25<PIC<0.5) at two polymorphic loci in Sahiwal cattle. The parameters of linkage disequilibrium between the two SNPs loci in the *KCNB1* gene were D':0.290 and r^2:0.037. The difference between D' and r^2 can be explained in two ways. First, D' may have been biased by a low representation of minor alleles in our population, and second, r^2 was influenced by differing frequencies of linked alleles. The low r^2 in our studied population indicated that one SNP explains only a small proportion of the variation held by the second SNP.

Association analysis showed that no significant correlation was observed between different genotypes of SNPs loci viz. SNP1-*Msp*1 and SNP2-*BspH*I with clinical mastitis in the analyzed populations (P<0.05). In agreement with our results, Asaf *et al.* (2015) reported that there was no correlation between G22231T and mastitis incidence in Vrindavani cattle. Similarly, Daldaban *et al.* (2021) observed no relationship between G22231T and T25025A loci of BRCA1 gene, however, they stated that the AB genotype for the G22231T variant and CC genotype for T25025A variant was more prevalent than the other genotypes among the group of subclinical mastitis in Holstein cattle. Interestingly, the present study revealed a significant (P<0.05) association with the production trait that was detected in the SNP1-*Msp*1 (78216220G>A) locus. At the SNP1-*Msp*1 locus, the value of test day milk yield (TDMY) for GG animals was significantly superior to those with AA and GA animals. Similarly, at the same locus (SNP1-*Msp*1), distance between fore and rear teat (DFR) for individuals with GA genotype was observed significantly (P<0.05) superior to AA and GG genotypes, while genotype GG was found significantly (P<0.05) superior with teat diameter (TD) as compared to AA and AG genotypes. In addition, at the locus SNP2-*BspH*I

Table 4. Associations between the novel polymorphism of the *KCNB1* gene and teat type traits in Sahiwal cattle

SNPs		SNP1-*Msp*1 (g.78216220G>A)			SNP2- *BspH*I (g.78216335A>G)	
Genotypes		GG (20)	GA (29)	AA (38)	AA (44)	AG (43)
Teat traits	TC	8.32±1.17	9.52±.97	8.12±0.85	8.81±0.79	8.45±0.80
	FTL	6.50±1.34	8.05±1.12	6.19±0.97	7.24±0.91	6.51±0.92
	RTL	5.48±1.48	7.34±1.23	5.54±1.07	7.02±0.99	5.20±1.00
	DFR	4.76±1.12[a]	7.82±0.93[b]	5.46±0.81[a]	5.71±0.77	6.47±0.78
	DLR	6.05±1.21	8.44±1.01	6.83±0.88	6.95±0.82	7.43±0.83
	SDF	42.94±1.39	40.98±1.16	42.81±1.01	42.38±0.94	42.07±0.95
	SDR	42.90±1.41	42.01±1.17	44.04±1.02	43.57±0.95	42.62±0.96
	TD	28.25±1.10[b]	24.91±0.91[a]	25.83±0.79[ab]	26.52±0.75	25.62±0.76

TC, Teat circumference; FTL, Fore teat length; RTL, Rear teat length; DFR, Distance between fore and rear teat; DLR, Distance between the left and right teat; SDF, Shortest distance of floor from fore teat; SDR, Shortest distance of floor from rear teat; TD, Teat diameter. Number of animals is indicated in parenthesis; values with distinct superscripts differ significantly.

(g.78216335A>G), the genotype AA was demonstrated superior to traits of rear udder height (RUH) and rear udder width (RUW) as compared to AG genotype in Sahiwal cattle.

These results are in concurrence with the findings of Strillacci *et al.* (2014), who detected the polymorphic locus at the same site, namely, SNP rs41710487/ SNP1-Msp1, and this locus was associated with milk yield, udder attachment, udder depth, udder height, and udder width in Valdostana Red Pied cattle. Yadav and Mukherjee (2018) detected GC genotype at G649C locus of FABP3 gene was superior concerning of FL305DPY and FL305DFY traits in Sahiwal cattle. Moreover, Kolbehdari *et al.* (2008) detected SNP at 81.7 Mb with the trait of the mammary system, which appeared to be associated with multiple udder traits. In addition, Cole *et al.* (2011) reported SNP in the renin gene (REN) of BTA16 and an SNP in the phosphorylase kinase, alpha 2 (PHKA2) gene of BTAX were most significant for udder and teat type traits in U.S. Holstein cows. In crossbred (*Bos indicus* × *Bos taurus*) cows, Tolleson *et al.* (2017) identified fifteen SNP loci on BTA 5 that were associated with udder support and average teat diameter. Furthermore, Sun *et al.* (2021) recently revealed that in Chinese Holstein cows, the SNP g.12264 C>T of the AGPAT3 gene is favorably correlated with test-day milk yield, protein %, and 305-day milk yield. Likewise, In Chinese Holstein cows, Li *et al.* (2021) discovered that the SNP loci c.908 C>T and c.1571 G>A of the FADS2 gene were substantially related ($P<0.01$) with test-day milk yield, fat percentage, and 305-day milk, and protein yield. In addition, Hosseini *et al.* (2021) detected g.304050G>A and g.325997 G>A SNPs loci of PPARGC1A gene were associated with milk yield and protein percentage in Italian Mediterranean buffalo.

In conclusion, to our knowledge, this is the first study to assess the effects of *KCNB1* polymorphisms on production characteristics in Indian Sahiwal cattle. The GG genotype at the SNP1-Msp1 (g.78216220G>A) locus was the most desirable one to select for in animals because it was connected with considerably greater test day milk production and udder type characteristics. Further study is needed before using SNPs in marker assisted selection to determine how they affect production-related characteristics in other populations and breeds.

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