



Lack of association of allelic variants of BRCA1 gene with mastitis susceptibility in Vrindavani cattle

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Pathogens, host genetics, management, hygiene and health of the dairy cattle affect the occurrence of mastitis (Zhang *et al.* 2009). Tolerance to mastitis showed that genetic variation exists among animals and breeds, with indigenous breeds being naturally less susceptible. Genetic marker assisted selective breeding has now become widely accepted (Wang *et al.* 2011) and single nucleotide polymorphism (SNP) markers are a great choice. Breast cancer 1, early onset gene (BRCA1) or breast cancer type 1 susceptibility protein homolog located on chromosome 19 (BTA19) is considered as one of the most potent genes influencing SCC and mastitis. There has been very little study regarding the SNP polymorphisms of bovine BRCA1 gene in relation to mastitis even though this gene has been studied in various species for various other pathologies like breast cancer, and related researches (Yuan *et al.* 2012). Hence, in this study, we hypothesized polymorphism at the SNP region contributes to resistance in mastitis. The objective of this experiment was to identify the SNP variant G22231T of bovine BRCA1 gene and to investigate the association of it with mastitis in Vrindavani crossbred cattle.

Blood samples were collected randomly from 98 lactating Vrindavani crossbred cattle (Holstein Friesian/Brown Swiss/Jersey × Harijana) maintained at cattle and buffalo farm of Institute. The animals were grouped into mastitis unaffected (66) and affected animals (32) on the basis of California mastitis test (CMT) and somatic cell count (SCC). The cows which had never been affected by clinical mastitis during their productive life (obtained from health records) and tested negative for California mastitis test (CMT) and somatic cell count (SCC) were kept in the mastitis unaffected group. Whereas, the cows affected with clinical mastitis at least once on the basis of history of animals, positive for CMT (CMT score >2) and SCC (more

than 3 lakh cells /ml) were kept in the clinical mastitis affected group. Genomic DNA was isolated from blood samples using phenol-chloroform extraction method. PCR-RFLP technique was used for SNP genotyping of the 98 animals. The primers used for amplifying the 321bp PCR product were 5'-CTTCAGAACCTGTACTTGTAACC-3' (forward) and 5'-CAAGGAATATTTACTGAGCACC-3' (reverse) which were reported earlier (Yuan *et al.* 2012). PCR reaction was carried out in a final volume of 20 µl reaction mixture containing 10.55 µl of nuclease free water, 4 µl of 5X Green GoTaq®Flexi buffer, 1.75 µl MgCl₂ solution (1.5 mM), 0.5 µl of PCR nucleotide mix (0.2 mM), 0.5 µl each of upstream and downstream primers (10 pmol/µl), 0.2 µl of Taq DNA polymerase (5U/ µl) and 2 µl of template DNA (40–50ng/ µl). PCR profile consisted of initial denaturation at 95°C for 5 min, followed by 32 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 30 sec, extension at 72°C for 30 sec with final extension for 10 min at 72°C. Aliquots of 5 µl PCR amplified products were digested with 2U of *HhaI* at 37°C for 3 h. The RE digested products were detected by electrophoresis in 2.5% agarose gel stained with ethidium bromide electrophoresis for 1 h in 1X TBE buffer. The gene and genotype frequencies of different patterns were estimated by standard procedure (POPGENE version 1.32) and for the statistical analysis, logistic regression methods were fitted using STATA 12 software. Initially in the analysis, the non-genetic factors like age at first calving, lactation length and calving interval were also fitted and we found none of these effects were significantly affecting the incidences of mastitis. Hence, further these non genetic factors except milk yield at 300 days were dropped in our model fitted for studying effect of different genotypes on mastitis. The following model was used for the analysis of the data;

Define $Y_i = 1$ if individual i is mastitic, 0 otherwise; A , the intercept; B , regression coefficients for different parameters; X_{1i} , dummy variable for genotypes of BRCA1 fragment (2 genotype groups i.e. 1 for AA and 2 for AB); X_{2i} , variable for milk yield as continuous covariate;

Then, our model is $Y_i \sim \text{Bernoulli}(\pi_i)$, where

$$\ln(\pi_i/1-\pi_i) = \alpha + \beta_1 X_{1i} + \beta_2 X_{2i} + \epsilon$$

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Table 1. Data of gene, genotype frequencies, average heterozygosity, PIC and χ^2 values

Locus	Allele A	Allele B	Genotype AA	Genotype AB	Genotype BB	Average. Het.	PIC	Chi-square
Mastitis negative animals (65 animals)								
BRCA1 G22231T	0.5923	0.4077	0.18	0.82	0	0.4830	0.3663	30.14
Mastitis positive animals (33 animals)								
BRCA1 G22231T	0.6061	0.3939	0.21	0.79	0	0.4775	0.3635	13.33

BRCA1 is involved in transcriptional regulation, DNA damage repair, cell cycle regulation and other important pathway to inhibit tumour and make sure of the maintenance of genome stability (Xu *et al.* 2012). BRCA1 is located within or nearby the genomic region of SCS quantitative trait loci (QTL) (Daetwyler *et al.* 2008). BRCA1 has been primarily studied in relation to breast cancer (Mahfoudh *et al.* 2012, Xu *et al.* 2012) but recently its role in mastitis has gained attention. Utilizing polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and created restriction site PCR (CRS-PCR) methods, three SNPs on bovine BRCA1 gene were studied and found significant association in the SNP C28300A (Yuan *et al.* 2012). Although introns were not coding sequence, evidences were provided constantly to prove that introns played an important role on regulating mRNA splicing, transcription and gene expression and regulation. Hence, in the current study, we focussed our analysis on a 321bp fragment of BRCA1 located on intron 6 spanning SNP variant G22231T. The animals were genotyped based on the electrophoretic pattern of RE digested PCR product. Those animals which have shown bands of 272bp and 49bp

Table 2. Effect of BRCA1 G22231T genotype and other variables on mastitis incidence

Mastitis	Odds ratio	Std. Err.	P value
Genotype AB	3.517554	1.465247	0.073
300 days milk yield	1.000286	0.0002532	0.258
Cons.	0.0964023	0.1099287	0.040

were grouped as wild homozygous (AA) and those with 321bp, 272bp and 49bp were categorized as heterozygous (AB) (Fig.1). Surprisingly, there were no mutant homozygous animals among the population under study. The gene, genotype frequencies, average heterozygosity and PIC values for both the mastitis negative and positive animals for the locus BRCA1 G22231T are depicted in Table 1. Effect of BRCA1 G22231T genotypes and other variables on mastitis incidence are depicted in Table 2.

The Chi-square value for the locus indicated the population is not in Hardy Weinberg equilibrium. PIC is less than 0.5 indicates the locus is moderately polymorphic. The association of allelic variants with mastitis susceptibility was worked out by using logistic regression. In the present study we did not have enough evidence to reject the null hypothesis and conclude even though genotype AB with approximately 3.5 times higher odds of mastitis occurrence in comparison to genotype AA but is not significantly different. This finding is in terms with that of Yuan *et al.* (2012), where they observed AB genotype had the highest SCS than their homozygotes, but it was not significantly different.

SUMMARY

BRCA1 is a latest entrant as a candidate gene for mastitis tolerance which warranted further investigations in order to ascertain its effect on mastitis. To the best of the author's knowledge, only two studies were published to ascertain the association of BRCA1 SNPs and mastitis tolerance. In the present study, the genomic DNA was isolated from blood samples and PCR-RFLP was used to genotype one of the BRCA1 SNPs i.e. G22231T. The analysis yielded no significant association with mastitis susceptibility. However, to our surprise the said mutation was never observed in the homozygous state in the population studied, may be suggesting that homozygosity for these alterations is incompatible with life. Hence, further studies have to be undertaken in large population and in other SNP regions of this gene in order to validate the real impact of BRCA1 gene on mastitis.

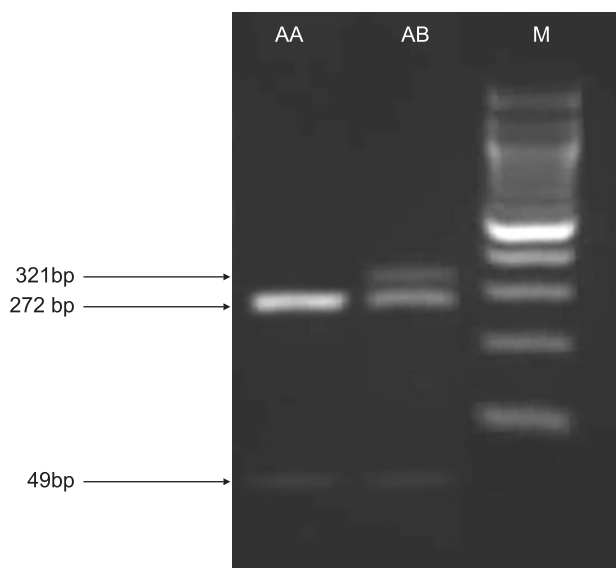


Fig. 1. Restriction profile of 321bp PCR products of bovine BRCA1 gene encompassing SNP variant G22231T. It is digested with *HhaI* enzyme. Lane M, 100bp DNA marker; lane 1, showing bands of 272bp and 49bp and grouped as wild homozygous (AA genotype); lane 2, showing bands of 321, 272 and 49bp and grouped as heterozygous (AB genotype).

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