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RESEARCH ARTICLE
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Association study of genetic variants at single nucleotide polymorphism rs109231409 of mannose-binding lectins 1 gene with mastitis susceptibility in Vrindavani crossbred cattle

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Abstract

Aim: The present study was undertaken to identify whether single nucleotide polymorphism (SNP) rs109231409 located on mannose-binding lectins 1 (*MBL1)* gene was associated with mastitis tolerance/susceptibility.

Materials and Methods: After grouping 100 Vrindavani crossbred cattle as mastitis positive and negative animals, they were genotyped using polymerase chain reaction (PCR)-restriction fragment length polymorphisms method. Gene and genotype frequencies of different patterns were estimated by standard procedure (POPGENE version 1.32, (University of Alberta, Canada) and statistical analysis was carried out by logistic regression methods using STATA 12 software (StataCorp LP, USA).

Results: The 588 bp fragment of *MBL1* gene was amplified using PCR. PCR product was digested with *ApaI* restriction enzyme showed two distinct genotypes viz., GG (311 bp and 272 bp fragments) and GA (588 bp, 311 bp and 277 bp fragments). The gene, genotype frequencies, average heterozygosity, polymorphic information content and χ^2 values for the locus rs109231409 was ascertained.

Conclusions: No significant association between SNP "rs109231409" with mastitis tolerance was found. Although there is a lack of association, further studies have to be undertaken in a large population in order to validate the impact of rs109231409 (g.855G >A) on mastitis tolerance.

Keywords: mannose-binding lectins 1, mastitis, polymerase chain reaction-restriction fragment length polymorphisms, single nucleotide polymorphism.

Introduction

Bovine mastitis is an economically important inflammatory udder disease creating havoc to the dairy industry worldwide [1]. Mastitis can be caused due to microbes like bacteria, viruses, mycoplasma, yeasts and algae [2] or can be due to thermal, chemical or mechanical injury [3]. Bacterial mastitis is the most common [4] which can be due to contagious pathogens or environmental pathogens [5]. Development of animals tolerant to mastitis is always sought after by researchers owing to the economic importance of the disease. Mastitis tolerances being a threshold trait; the selection strategies are a little different from the conventional approaches for quantitative traits. Lack of phenotypic data on clinical mastitis underscores breeding programmes and hence heavily relies on somatic cell count (SCC) as an indirect indicator for clinical mastitis [6].

California mastitis test (CMT) is a simple and inexpensive test that gives qualitative estimate of SCC in the foremilk of individual cows or quarters. CMT is often used as an indirect measure of mastitis [7,8] but SCC is regarded as the gold standard to measure mastitis, and other methods are compared with SCC [9]. Milk SCC has been used extensively as an indicator of intra-mammary infections since 1960. This trait is used as an indicator of udder health for management and selection purposes [10]. SCC is a fast and reliable analytical tool. It is related to the immunological status of the udder and increases in response to an inflammatory stimulus like bacterial infection [11,12]. Currently by many researchers across the globe has used SCC for mastitis resistance studies [13-15]. SCC and mastitis are genetically correlated on an average 0.7 [16].

There are a number of candidate genes associated with mastitis tolerance of which Mannose-binding lectins 1 (*MBL1)* gene requires special attention. The bovine *MBL1* gene is located on chromosome 28 [17]. Most of the mammals have two *MBL* genes viz., *MBL1* and *MBL2*. *MBL1* gene encodes the *MBL-A* protein. *MBL1* gene is involved in the innate immune response mediation process [18]. Recently, single nucleotide polymorphism (SNP) in the *MBL1* gene has been searched for its association with mastitis tolerance,

and a few of them was found to be associated. All these studies were conducted in Chinese native cattle. There is a need to check whether these SNPs have any association in crossbred cattle of India in an attempt to identify these SNP markers which can be incorporated into the breeding programmes in future.

The current study was undertaken to identify whether the SNP rs109231409 (g.855G>A) located in the introns 1 of *MBL1* gene was associated with mastitis tolerance or not.

Materials and Methods

Ethical approval

The experimental and plan of study was duly approved by Institution Animal Ethics Committee of Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India.

Resource population

The experimental study consisted of 100 Vrindavani crossbred cattle (Holstein Friesian/Brown Swiss/Jersey X Haryana) maintained at cattle and buffalo farm, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India.

Grouping of animals

One hundred Vrindavani milch animals were selected randomly. These animals were then grouped into mastitis negative (65) and positive animals (35) on the basis of CMT along with SCC.

Genotyping of SNP

The SNP "rs109231409" located on the *MBL1* gene was genotyped using polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP). Genomic DNA was isolated from blood samples using phenol-chloroform extraction method. Primers for the PCR reaction for amplifying the introns 1 of *MBL1* gene was 5'-CCCTTCCAACTCATTGCTTC-3' (Forward) and 5'-AGTCCCAACCACCCTCA-3' (Reverse). The PCR reaction was carried out in a final volume of 25 µl reaction mixture containing 12.5 µl of GoTaq Green MasterMix (Promega, Madison, USA) and 1.25 µl each of upstream and downstream primers (10 pmol/µl). PCR profile consisted of initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 94° for 30 s, annealing at 62°C for 30 s, extension at 72°C for 30 s and final extension for 5 min at 72°C. The amplified PCR products were then subjected to restriction enzyme (RE) digestion using *Apa*I enzyme. Aliquots of 20 µl PCR amplified products were digested with 10U of *Apa*I (New England Biolabs, Massachusetts, USA) at 25°C for 3 h. The RE digested products were detected by electrophoresis in 2.5% agarose gel electrophoresis stained with ethidium bromide for 1.5 h in ×1 TBE buffer.

Statistical analysis and association study

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 (StataCorp LP, USA).

Mastitis affection of animals was taken as dependent variable whereas genotypes of the fragment was considered as independent variables. Initially in the logistic regression analysis, factors like milk yield, age at first calving, lactation length and calving interval were also fitted but none of these effects was significantly affecting the incidences of mastitis. Hence, these variables were dropped in the model fitted for studying effect of different genotypes on mastitis. The following model was used for the analysis of the data;

Define Yi=1 if individual i is mastitic, 0 otherwise;
α=The intercept
β=Regression coefficients for different parameters
X1i=Variable for genotypes of *MBL1* fragment (2 genotype groups i.e. 1 for GG and 2 for GA).

Then, our model is Yi ~ Bernoulli (pi), where
$\text{Ln}(pi/1-pi)=\alpha+\beta 1X1i+\varepsilon$

Results

The 588 bp fragment of *MBL1* gene amplified using PCR on digestion with *Apa*I RE yielded two distinct genotypes viz., GG (311 bp and 272 bp fragments) and GA (588 bp, 311 bp and 277 bp fragments) (Figure-1). The gene frequency of allele G was found to be 0.7462 and 0.7286 and that of allele A was 0.2538 and 0.2714 respectively in mastitis positive and negative animals. Polymorphic information content (PIC) for both mastitis positive and mastitis

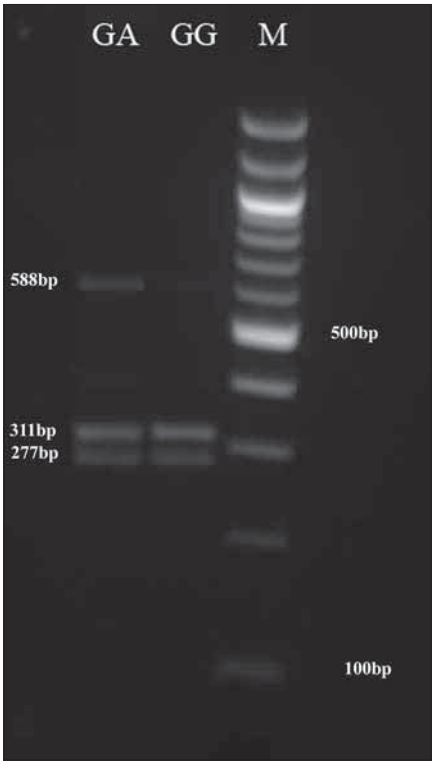

Figure-1: Polymerase chain reaction-restriction fragment length polymorphisms of mannose-binding lectins gene fragment using *Apa*I RE. (Lane 1: GA genotype, Lane 2: GG genotype, Lane 3: 100 bp DNA ladder).

negative were found to be <0.5 indicating that the locus is moderately polymorphic in Vrindavani herd. The population under study was not found to be in Hardy Weinberg equilibrium as there was a significant difference between observed and expected frequencies of genotypes ($p<0.05$). In the present study, we did not find enough evidence to reject the null hypothesis ($p=0.737$) and conclude the odds of mastitis occurrence in genotype GA compared to genotype GG is not significantly different. The gene, genotype frequencies, average heterozygosity, PIC and χ^2 values for the locus rs109231409 is shown in Table-1.

Discussion

MBL1 gene contains four introns and five exons, encoding a 248 aa protein. *MBL* is a pattern recognizing serum protein that participates in the innate immune system of mammals as an opsonin. The antimicrobial function of *MBL* includes opsonization, neutralization and complement activation [19]. The recognition domain of *MBL* selectively targets invading microorganisms by binding to mannose and N-acetylglucosamine residues on the cell surface and thereby activating *MBL*-associated serine proteases (MASPs) [20]. *MBL* circulates in complex with MASPs MASP-1, MASP-2, MASP-3 and MAp19. When *MBL* recognizes suitable patterns, results in activation of MASPs initiating the *MBL* pathway of complement activation leading to opsonization or direct killing of targeted microorganisms [21].

MBL1 is a critical gene in relation to the innate immune response of the animal. *MBL*s have been found to be associated with susceptibility to various bacterial and viral diseases [22]. *MBL1* in the porcine and bovine is considered as a candidate gene for mastitis resistance [23]. *MBL1* gene was proposed as an indirect marker to improve dairy mastitis resistance traits in cattle [24]. SNPs of *MBL1* gene in various breeds of pigs were found to be associated with impaired disease resistance [25]. Both *MBL1*and *MBL2* gene mutations were found to be having impact on susceptibility to different infections [26]. Variations in the coding and the non-coding regions of the *MBL* gene in various species resulted in innate immune dysfunction [23]. The SNP under study, rs109231409 (g.855G>A) is located in the intron I and is characterised by G>A mutation. Association study of rs109231409 in Chinese native cattle also yielded a similar result that they could not find any significant association with mastitis tolerance [27]. In that study, they screened three SNPs in the *MBL1* gene with mastitis tolerance and found that no association between g.855G>A or g.2686T>C but significant association was found for g.2651G>A. On analysis of four SNPs on *MBL2* gene in Chinese Holstein cattle and Luxi yellow cattle, the authors found two of them viz., g.201 G > A, and g.234 C > A to be significantly correlated with somatic cell score [23].

Conclusion

In the present study, 100 randomly selected Vrindavani crossbred cows were grouped into mastitis positive (35), and negative animals (65) based on CMT and SCC. Genomic DNA was isolated by using phenol-chloroform extraction. PCR-RFLP technique was used to genotype the SNP rs109231409 (g.855G>A) located on the *MBL1* gene. The 588 bp PCR product was digested with *Apa*I RE to yield two distinct genotypes viz., GG (311 bp and 272 bp fragments) and GA (588 bp, 311 bp and 277 bp fragments). The locus was found to be moderately polymorphic. Statistical analysis yielded no significant association between the genotypes with mastitis tolerance. Although there is a lack of association, further studies have to be undertaken in a large population in order to validate the impact of rs109231409 (g.855G>A) on mastitis tolerance.

Authors' Contributions

VNMA, BB and MP: Substantially contributed to design and plan of the study. VNMA, BB and MP: Drafted the manuscript, analysed and interpreted the results. VNMA, BB, MP, PD, AK, PK and GKG: Revised manuscript for important intellectual content. All authors read and approved the final manuscript.

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

Locus	Allele G	Allele A	Genotype GG	Genotype GA	Genotype AA	Average. Het.	PIC	Chi-square
Mastitis negative animals (65 animals)								
MBL1 rs109231409	0.7462	0.2538	0.49	0.51	0	0.3788	0.307	7.257732
Mastitis positive animals (35 animals)								
MBL1 rs109231409	0.7286	0.2714	0.46	0.54	0	0.3955	0.3173	4.560

MBL1: Mannose-binding lectins, PIC: Polymorphic information content

Institute under the project "Genetic variability of host resistance to mastitis in cattle."

Competing Interests

The authors declare that they have no competing interests.

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