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Characterization and Validation of Point Mutation in Breast Cancer 1 (BRCA1) and Its Relationship with Mastitis Traits in Sahiwal

Ankit Magotra[1], I.D.Gupta[2], Archana Verma[2], Rani Alex[3], Vijay Kumar[4], M.R. Vineeth[5], Ashwani Arya[5], A. Sakthivel Selvan[5]

[1]Assistant Professor, Department of Animal Genetics and Breeding, LUVAS, Hisar-125004 Haryana, India
[2] Principal Scientist, Dairy Cattle Breeding Division, ICAR- National Dairy Research Institute-132001
[3]Scientist, Central Research Institute on Cattle, Meerut, Uttar Pradesh
[4] Assistant Professor, Department of Animal Genetics and Breeding, U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh
[5]PhD Scholar, Dairy Cattle Breeding Division, ICAR-National Dairy Research Institute-132001

Abstract: **Bovine mastitis is a very common and multietiological disease of dairy cattle which leads to huge economic losses to the dairy industry globally. In this study, the bovine breast cancer 1, early onset gene (BRCA1) was taken as a candidate gene for mastitis resistance. Breast cancer 1 (BRCA1) is one of the genes which predispose organism to early-onset breast cancer, and is involved in DNA damage repair, cell cycle regulation, transcriptional regulation, and other important pathways to suppress tumor and maintain genome stability. A total of 120 Sahiwal cattle were selected to characterize the targeted region of intron 6 of BRCA1 gene for polymorphism screening and their association with mastitis. A 321bp PCR fragment of BRCA1 gene encompassing the targeted region of intron 6 was amplified and digested with *Hha* I to screen for the reported SNPs having significant association with SCS. Genotype analysis using PCR-RFLP revealed a monomorphic banding pattern. Sequencing was also carried out to explore the *in silico* screened SNPs which are deposited in dbSNP. The result indicates highly conserved sequence in Sahiwal cattle. Therefore, reported as well as *in silico* SNPs cannot be considered as a universal marker for mastitis in all the breeds. Since, present study has formulated the results based on a relatively small sample; further studies are required to screen these SNPs in large samples.**

Keywords: **Sahiwal, Polymorphism, BRCA1, Mastitis, PCR-RFLP.**

1. INTRODUCTION

India is bestowed with a huge livestock population of 512.05 million [1] which spread over a total geographical area of 3,287,240 sq km, with a major proportion of biodiversity including some of the excellent breeds of cattle and buffalo known so far. Some of the states are truly regarded as a hub of the dairy contributors to the national pool because of excellent dairy breeds of cattle and buffalo they have, and constitute only 1.3% of the country's geographical area. Beneficial animal production for dairy farmers is about implementing sound practices on dairy. Good dairy farming practices ensure that the milk is produced by healthy animals in a sustainable manner and are responsible for the animal welfare from social, economic and environmental perspectives. Despite the fact that India is a global leader in milk production, our per capita yield is lowest. The major concern of country is the low productivity of indigenous animals and production related diseases in high yielding cattle which has huge economic consequences. Among the several bottlenecks in achieving the milk production targets, mastitis continues to remain as the most challenging impediment [2]. The prevalence of mastitis is also increasing corresponding with the increase in milk production. Based upon several recent studies statistics on mastitis prevalence across different states in India, it was observed that it ranges from 25-97% with an

average prevalence of 45% [3]. Mastitis is a very complex and common disease of dairy cattle, which causes a major economic loss to the dairy industry worldwide [4]. Mastitis is an inflammatory reaction of mammary gland parenchyma caused by bacteria and their toxins. It is characterized by physical, chemical and usually bacteriological changes in the milk and by pathological changes in the glandular tissue. Mastitis primarily results from invasion of pathogenic organisms through the teat canal, resulting in loss of potential milk production in the affected quarter of the gland [5]. In India, there is a rampant increase in the economic losses due to mastitis which increased about 115 folds in the last five decades from Rs. 529 million per annum in 1963 [6] to Rs. 7165.51 crore per annum in 2012 [7].

Research on mastitis vaccines has been conducted for four decades and several mastitis vaccines are commercially available [8]. However, it is difficult to prevent mastitis in all the locations globally with these few vaccines because mastitis is a multietiological disease, and available vaccines may not be sufficient to contain the threat. In India, genetic selection is merely on increased milk production, but the unfavourable correlations between milk yield and clinical mastitis (CM) suggest that selection solely for milk yield will increase the CM incidence. This effect can be counteracted by simultaneous selection on higher milk production and mastitis resistant animals. Genetic studies of records on veterinary treatments in the Nordic countries have shown that it is possible to improve the disease resistance by selective breeding [9]. A number of candidate genes with physiological effects on disease resistance traits are being explored for their possible roles in the control of expression of varying phenotypes observed in different breeds or within breeds [10, 11]. Breast cancer 1 (BRCA1) is one of the genes that confers genetic predisposition to early-onset breast cancer, and works in the process of DNA damage repair, cell cycle regulation, transcriptional regulation, other important pathway to ultimately suppress tumor and maintain genome stability. The bovine BRCA1 gene has been mapped to chromosome 19 (BTA19) [12]. This location was within a region of similar gene order as the BRCA1 locus in human chromosome 17 and mouse chromosome 11 [12, 13]. Many researches pointed out that mutations in the gene encoding[12, 13] BRCA1 were associated with a high risk of breast cancer, and related researches have been reported mainly in human and other model animals [12, 14-16]. The information on genetic polymorphism of BRCA1 and their association with mastitis using Somatic Cell Score (SCS) has been reported in *Bos taurus* cattle [17, 18] but so far, no research has been carried out in *Bos indicus* cattle.

2. MATERIALS AND METHODS

Animals and DNA preparation:

The analysis was performed on 120 cattle represented by Sahiwal breed. All animals were maintained at the cattle yard of National Dairy Research Institute, Karnal, India. Animals which were not affected up to third lactation were taken as control. Genomic DNA was extracted from blood by protocol of [19]. Quality and quantity of the isolated genomic DNA was evaluated using UV-vis spectrophotometer (Biophotometer Plus, Eppendorf).

Primer design and PCR amplification:

Based on reference sequence (ENSBTAG00000022520) of the bovine BRCA1 gene, specific PCR primers were designed using Premier 5.0 software to amplify targeted region of intron 6 of BRCA1 and verify candidate SNPs in this genomic region. PCR amplification was carried out in a total volume of 25 µl with 100 ng DNA template, 1x PCR buffer, 1.5 mM MgCl2, 200 µM of each dNTPs, 20 pmol of each primer and 1 unit of Taq DNA polymerase. PCR was carried out in thermal cycler (T-100 Bio Radd) in following stages – initial denaturation at 92°C for 5 min., followed by 35 cycles of 94°C for 30 s., annealing at 54.5 °C for 30 s, 72°C for 30 s and a final extension at 72°C for 5 min. The PCR products were separated on 1.5% agarose gel including 0.5 µg/ml of ethidium bromide, and then photographed under UV light.

Genotyping G43761121A SNP:

In this study, candidate SNP viz., G43761121A in intron 6 of BRCA1 gene was targeted and genotyped by PCR RFLP and DNA sequencing methods in Sahiwal cattle (Reference sequence: ENSBTAG00000022520). The genotyping of animal for reported G43761121A candidate SNP for SCS [10] was performed through PCR-RFLP. Amplified PCR products (10 µl) were digested with 2 U *Hha I* restriction enzyme at 37°C for 10 h. The digested product was separated on 2.5% agarose gel and the gel was stained with ethidium bromide. The PCR amplified products of 20 samples (10 individuals from both affected and non affected groups selected randomly) were sent to the 1st base Molecular biological services (Malaysia) for purification and sequencing in both directions for genotype confirmation and characterization of

amplified region. The dbSNP database was also investigated in this study (http://www.ncbi.nlm.nih.gov/SNP/) for screening mutations in the bovine BRCA1 gene. The sequence was assembled by BioEdit software to screen for candidate SNPs.

3. RESULTS AND DISCUSSION

Dairy breeding programs were solely focused on improvement in the production traits with little emphasis on health traits. Despite the fact that many studies have made an effort to explore the nature of this problem globally, mastitis continues to be the expensive disease with huge economic losses. There are complications associated with therapeutic interventions involving resistance to antibiotics, efficacy and cost-effectiveness issues. Development of successful vaccines against mastitis remains an obstinate problem due to involvement of wide spectrum of etiological agents, lack of information on the genetic factors of disease resistance and complications associated with damage to mammary epithelial cell by both the agents and the host factors [20]. There has been growing amount of interest in selection for health traits in the dairy industry [10, 18]. The candidate gene approach may offer a more direct and comprehensive understanding of the genetic basis underlying the differences in the quantitative expression between different breeds.

BRCA1 gene is considered to be one of the potential candidate genes influencing SCS and mastitis. In the present study, we characterized partial intronic 6 region of BRCA1 gene in indigenous cattle (Sahiwal) to explore a candidate G43761121A SNP associated with SCS) [18] as well as other SNPs screened from dbSNP in *Bos taurus*. In this study, intron 6 of the *Bos taurus* BRCA 1 gene was screened for 19 SNPs i.e. A43761359T, C43761357A/G, G43761356A, T43761341C, G43761305C, C43761262T, G43761202A, G43761197C, T43761173C, A43761172C, T43761170C, C43761146A, A43761141C/G, C43761122T, G43761121T, G43761103A, A43761099C, A43761092C, A43761079C by using bioinformatics tools. The PCR amplification generated a targeted 321 bp intron 6 region of BRCA1 gene (Figure 1). The PCR products of animals under both groups i.e affected (60 samples) and non-affected (60 were digested with *Hha I* enzyme and resolved into monomorphic pattern GG in Sahiwal) are shown in Figure 2. Chromatograph as shown in Figure 3 also revealed conserved G allele at particular position in indigenous cattle. Multiple sequence alignment using ClustalW (Figure 4) revealed that the amplified BRCA1 nucleotide sequence from *Bos indicus* (Sahiwal) as well as the sequence corresponding to amplified region of BRCA1 gene from *Bos taurus* (Gene Id ENSBTAG00000022520) are in consonance. Thus, the animals under study were found to be monomorphic, which is reported first time in Sahiwal cattle, a finding which is contrary to previous reports of association between BRCA1 and mastitis. [17] reported three genotypes i.e. GG, AG and AA in this SNP region in Chinese Holstein breeds of cattle. On the other hand, [21] who reported two genotypes AA and AG in Frieswal (HF × Sahiwal) cattle showed significant association of CACNA2D1 gene with SCS. The monomorphic pattern observed in Sahiwal cattle for G43761121A SNP in BRCA1 gene with an aim to explore its possible association with SCS may be a breed specific characteristic. So, it is strongly suggested to explore the variation in different breeds before implementation in selection criteria.

4. CONCLUSION

This study seeks to unravel the association of BRCA1 gene polymorphism in indigenous cattle (Sahiwal) to mastitis at molecular level aimed at exploring the potential of G43761121A SNP to be utilized as a universal market for mastitis trait. We found no significant association of the candidate SNP with mastitis resistance, which maybe a breed specific characteristic. All animals were found to be monomorphic with respect to *in silico* and reported SNPs and the amplified genomic region was observed to be highly conserved. Therefore, reported as well as, *in silico* SNPs cannot be deemed to be a universal marker for mastitis resistance in all the cattle breeds. Since present study has formulated the results based on a relatively small sample, further studies are required to screen these SNPs in large samples to establish the role of SNPs in BRCA1 gene in conferring resistance against mastitis.

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APPENDIX - A

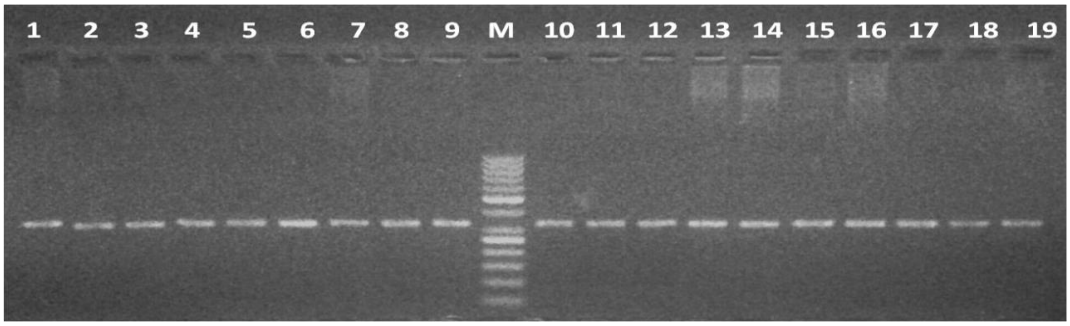

Figure 1: PCR amplified product of intron 6 in BRCA 1 gene in Sahiwal cattle

Lane 1-19 : Sahiwal PCR product (321bp)
Lane M : 50 bp DNA Ladder

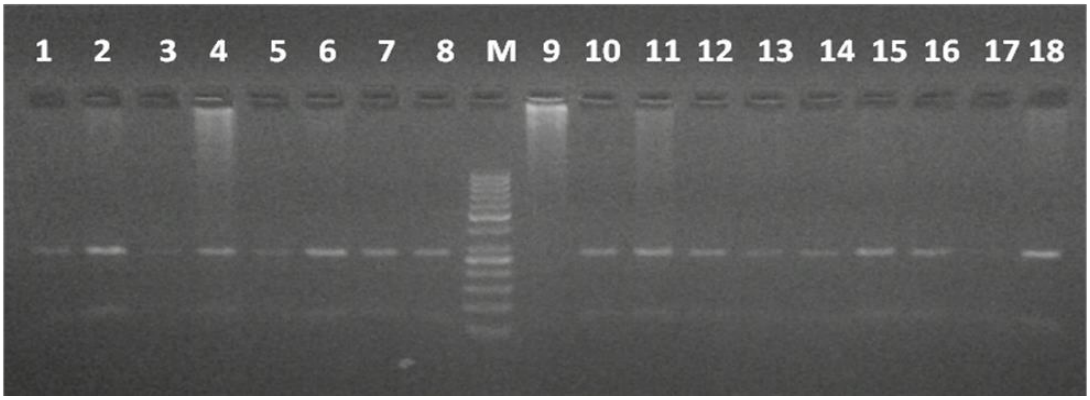

Figure 2: PCR-RFLP of intron 6 of BRCA1 gene in Sahiwal cattle using *Hha I* restriction enzyme

Lane 1-18 : 272, 49 bp GG genotype (Monomorphic)
Lane M : 50 bp DNA ladder

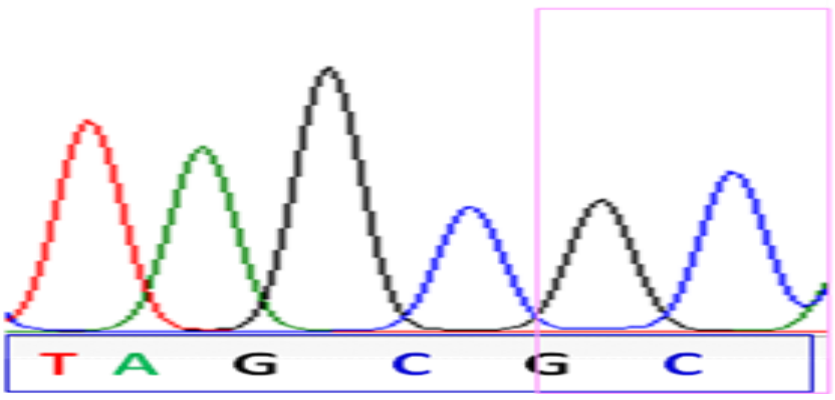

Figure 3: Chromatogram showing G allele at position G43761121A in intron 6 of BRCA1 gene in Sahiwal cattle

Figure 4: Sequence comparison of intron 6 of BRCA1 gene in *Bos indicus* (Sahiwal) against *Bos taurus* for primer design. Stars

Bos taurus	**TTCATTGGTGGGTGTGTTCTTTACCACTAGCGCCACCTGGGAAGCCCTGCCATGTACTGG 300**
Sahiwal	**TTCATTGGTGGGTGTGTTCTTTACCACTAGCGCCACCTGGGAAGCCCTGCCATGTACTGG 300**
	**********

denote identities between two sequences. Both sequences reveal 100 % identity.