

Genetic polymorphism of promoter region of lactoferrin gene and its association with mastitis resistance in Jersey crossbred cattle

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Received: 21-11-2013

Accepted: 26-09-2014

DOI: 10.5958/0976-0555.2015.00109.0

ABSTRACT

Lactoferrin, a protein found in variety of body secretions, acts as a defense factor in the mammary gland. Total of 50 samples of blood and milk of Jersey crossbred cattle were collected from the Government Dairy Cattle farm, Belicharana. Genomic DNA was isolated and specific primer was used for the amplification of the promoter region of lactoferrin gene. The PCR product comprised of 115 bp. The PCR product was digested with *Taq* I restriction enzyme. The amplicon has no restriction site and all the 50 animals revealed only one type of genotype (HH) with respect to *Taq* I / PCR-RFLP. The same PCR products were subjected to SSCP. Amplicons were initially denatured with denaturing agents and then run on native PAGE which was later silver stained. All the 50 samples revealed one type of banding pattern viz., two bands. Single genotype was present in all animals. Hence promoter region of Lactoferrin gene is monomorphic in crossbred cattle under the present study. Somatic cell count was done by staining milk samples with Newmans stain. A total of 19 milk samples had somatic cell count of less than 1,00,000 cells/ml with no history of mastitis were considered as resistant, while other 20 samples having somatic cell count more than 5,00,000 cells/ml with mastitis were considered as susceptible. Though variation in response to mastitis resistance was detected, no association was established. The reason being that promoter region of lactoferrin gene was monomorphic with respect to *Taq* I / PCR-RFLP and PCR-SSCP.

Key words: Crossbred cattle, Lactoferrin gene, Polymorphism, Promoter, SSCP.

INTRODUCTION

Mastitis is one of the major impediments in achieving increased milk production. Mastitis in dairy animals causes havoc on account of involvement of high treatment cost, milk disposal due to huge bacterial load, culling of animals and reduction in milk production. In India annual economic loss due to mastitis in cattle and buffalo was Rs. 2809.32 crores (Sirohi and Sirohi, 2001). Lactoferrin (Lf) seems to be an important candidate gene for mastitis resistance trait (Seyfert *et al.*, 1996). The identification of genes that have significant association with the mastitis would facilitate the inclusion of mastitis resistance in cattle breeding programmes.

The genetic variations present in the bovine Lf gene have been successfully associated with the mastitis susceptibility / resistance, and to its indicator trait i.e. somatic cell count and somatic cell score (SCC and SCS) in crossbred cattle. Somatic cell count (SCC) in milk constitutes a good diagnostic tool that allows early detection of either subclinical

or acute form of mastitis (Green *et al.*, 2004). Somatic cell count of milk is an important marker to detect clinical and sub-clinical mastitis (Beaudeau *et al.*, 2002).

Polymorphisms in the promoter region of Lf gene may lead to different allelic variants, which can have different levels of antibacterial and anti-inflammatory properties of the gene. Variation in promoter or regulatory region of Lf gene leads to variable expression of Lf protein in milk. Thus, it is imperative to explore the genetic polymorphism of Lf promoter in the cattle. Keeping in view of the above facts the present study has been conducted to explore the genetic variations in the promoter region of Lf gene in crossbred cattle.

MATERIALS AND METHODS

The present study was carried out at Division of Animal Genetic & Breeding on Jersey crossbred cattle maintained at Government cattle farm at Belicharana, Satwari, Jammu. A total of 50 animals were taken for study. Both blood and milk samples of these crossbred cattle were collected

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and taken under study to screen Lf gene polymorphism and to associate with mastitis through somatic cell count (SCC). 5 ml of blood was collected from jugular vein of cross bred cattle in a 15 ml polypropylene tube containing 250µl of 0.5 MEDTA as anticoagulant. Genomic DNA was isolated using standard phenol chloroform extraction method (Sambrook and Russell, 2001).

A pair of primers for amplification of promoter region of Lf gene were used on the basis of latest published sequences as reported by (Huang *et al.*, 2010) where single nucleotide polymorphism (SNP) in Lf gene was associated with mastitis. The forward primer was of 24 bp (5'-GAAGTCCTCCCCACCCCTTGTCG-3') and reverse primer was of 24 bp (5'AGGACACTCCCTTGAAGCACAACA-3') with annealing temperature 56°C. PCR was carried out in a final volume of 50 µl reaction mixture containing 100ng of template DNA, 1X PCR assay buffer, 2.0 mM of Mg²⁺, 200 µM of dNTPs, 10 pM of each primer and 1U of *Taq* DNA polymerase. Amplification was carried out in Thermal cycler (Eppendorf, USA). Different annealing temperature was tried to optimize PCR cycle condition. PCR condition were: initial denaturation at 94°C for 5 minutes; followed by 94°C for 40sec, 56°C for 40sec, 72°C for 1 min, and a final extension of 72°C for 5min.

In order to detect the PCR RFLP, the amplicon was digested with *Taq* I at 65°C. The restriction digestion was carried out in 25 µl volume. For setting a large scale restriction digestion a master mix was prepared on ice by adding the different components in following order: Assay Buffer C (10x) 2.5µl, *Taq* I enzyme (10U/µl) 1.5µl and autoclaved distilled water upto 25µl was taken. *Taq* I digested product was run on the 2.5% agarose gel, in 1 x TAE buffer for 2-3 hours at 5 V/cm.

The PCR products were subjected to non denaturing poly-acrylamide gel electrophoresis. 12% of acrylamide-bisacrylamide was prepared and the samples were run at 150V for 5 hrs. After completion of electrophoresis, the gels were stained by silver staining as described by Sambrook and Russell (2001) with slight modifications.

Total somatic cell count was done as per the modified technique of leukocyte count described by Prescott and Breed (1910). For association study animals were divided into two groups viz., affected and non-affected. The animal with SCC above five lakhs/ ml and signs of mastitis were grouped as affected. Whereas animals with SCC lower than one lakhs/ ml with no signs of mastitis till 3rd lactation or completion of the study, whichever is later were grouped as non-affected.

RESULTS AND DISCUSSION

Agarose gel electrophoresis of the amplicon revealed an amplification of a fragment of 115 base pair from the promoter region of Lf (Figure 1). Digestion of PCR product with *Taq* I revealed that there was no restriction site in the amplicon and all the 50 samples were of same genotype HH (only one amplicon of 115bp). However Huang *et al.* (2010) find three genotypes in the same region viz., GG with fragments 91bp and 24bp, GH with fragments 115bp, 91bp and 24bp and HH with fragment 115 bp. So in the present study no polymorphism with respect to *Taq* I / PCR-RFLP was observed. The SSCP analysis of promoter region of Lf gene revealed similar band patterns. There was no variation or polymorphism found in all the 50 samples. All animals had similar genotype. In present study, hence promoter region of Lf gene was found to be monomorphic with respect to particular primer as reported by Huang *et al.* (2010) in Jersey crossbred cattle. Based on somatic cell count and history of mastitis 19 animals were grouped as resistant and 20 as susceptible (Figure 2). All animals belonging to both resistant and susceptible group were of same genotype as no polymorphism was observed. Hence, association of mastitis resistance or susceptibility with promoter region of Lf gene could not be established.

The results were similar to reports of Li *et al.* (2004) which showed monomorphic patterns in Lf-5'2 and Lf-5'4

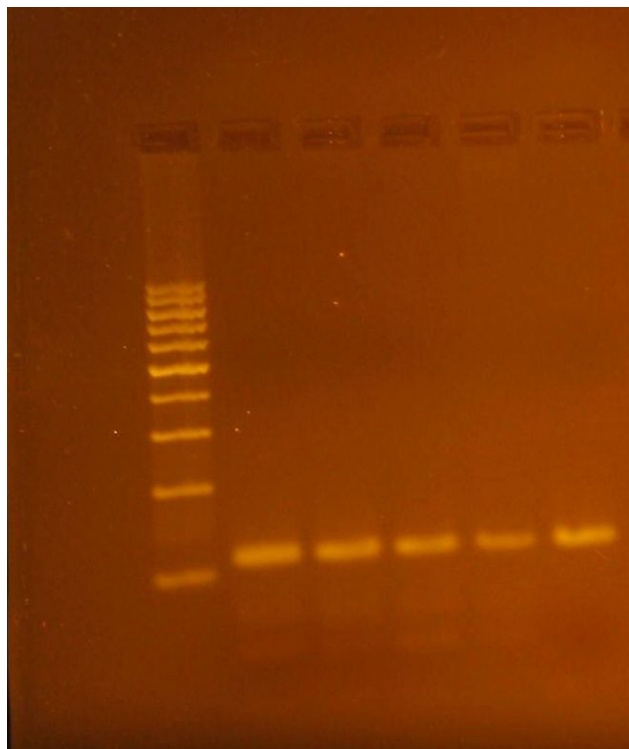


FIG 1: PCR product of 115 bp
Lane 1: 100 bp ladder
Lane 2-6: PCR amplicon

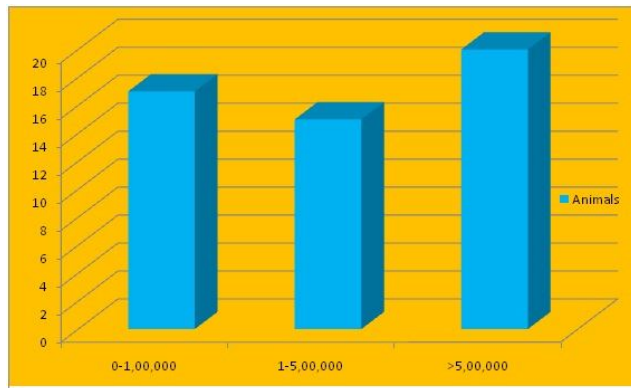


FIG 2: Somatic cell count

segment of promoter region of Lf gene in SSCP analysis. Monomorphism was also reported in exon 10, exon 13, exon 14 and exon 17 of Lf gene (*viz.*, two bands). However Lf 5'1, Lf 5'3 and Lf 5'5 segment of Lf 5' region showed polymorphic patterns.

Huang *et al.* (2010) explored single nucleotide polymorphism (SNP), haplotypes and combined genotypes of Lf and its association with mastitis in Chinese Holstein cattle. The result of haplotype analysis of four SNPs showed that fourteen different haplotypes were identified. Statistical analyses revealed no significant association between one single SNP of Lf gene and SCS, whereas significant associations between their combined genotypes of three SNPs, haplotype and SCS. Combined genotype EFCDBB

and GGEFDD with the lowest SCS were favorable for the mastitis resistance. However in the present study no association was found with mastitis resistance in Jersey crossbred cattle due to lack of polymorphism in promoter region of Lf gene.

Structure of promoter is the major region for regulating gene transcription. Polymorphisms in these regions can alter gene expression. Further study is needed to clarify the role of the genetic variants of Lf gene, and to analyze the mRNA expression levels of Lf gene. In the present study association of SSCP and *Taq I* / PCR-RFLP of promoter region of Lf gene with natural resistance to mastitis in Jersey crossbred cattle could not be established. However it would be interesting to study the Lf polymorphism in other breeds of cattle and buffaloes, and to identify other genes contributing to natural resistance.

CONCLUSION

Present study of Jersey crossbred cattle revealed that promoter region of Lf gene was monomorphic with respect to *TaqI*/PCR-RFLP and PCR-SSCP. Therefore, no association could be established with mastitis resistance, although, variation in response to mastitis resistance was detected. Future studies need to be directed to explore polymorphism throughout the entire Lf gene and to ascertain their suitability as potential class I marker for resistance against mastitis.

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