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SHORT COMMUNICATION

Lack of association of mastitis with allelic variants of *CXCR2* gene in Vrindavani cattle

M.S. Dige[a]*, S.P.S. Ahlawat[b], B. Bhushan[a], P. Kumar[a], B. Inamdar[c] and Amit Kumar[a]

[a]*Animal Genetics and Breeding Indian Council of Agricultural Research, Division of Animal Genetics, Indian Veterinary Research Institute, Bareilly, India;* [b]*Department of Genetics and Biostatistics, Veterinary College, Indian Veterinary Research Institute, Bareilly, India;* [c]*University of Agricultural Sciences, Raichur, Karnataka*

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The present study was aimed to identify single-nucleotide polymorphic (SNP) sites in coding regions of *CXCR2* gene in Vrindavani cattle. A total of 95 animals were screened for *CXCR2* gene locus. Single-strand conformation polymorphism (SSCP) analysis revealed polymorphic patterns, and a total of five distinct patterns were observed in the exon 2 of *CXCR2* gene. Sequence analysis of different SSCP variants revealed the presence of five novel SNP at A214G, T340C, G344A, C856T and G1027A. No association of mastitis incidence was detected with different patterns of SSCP for both amplicons of *CXCR2* exon 2.

Keywords: *CXCR2* gene; mastitis; polymorphism; Vrindavani cattle

Introduction

Many candidate genes for mastitis tolerance are being studied in different cattle populations. Genes associated with immune responses of mammary gland are potential genetic markers because of their importance in mastitis. Also, genes associated with neutrophil function are potential genetic markers for mastitis, as neutrophil migration from blood to the sites of infection is essential for resolution of most of the mastitis pathogens (Paape et al. 2000). One of the important chemokines associated with leukocyte migration is interleukin-8 (IL-8). IL-8 is an ELR^+ CXC chemokine, which interacts with specific chemokine receptors viz. *CXCR1* and *CXCR2* are present on the neutrophils surfaces (Lahouassa et al. 2008). These chemokines receptors are required for maximum neutrophil function during infection (Murphy & Tiffany 1991). Recognition of chemokines by *CXCR1* and *CXCR2* induces neutrophil activation, chemotaxis and, eventually, phagocytosis of pathogen (Podolin et al. 2002). Single-nucleotide polymorphisms (SNPs) have been identified in the bovine *CXCR1* found to be associated with mastitis resistance (Leyva-Baca et al. 2008). A few literatures (Youngerman et al. 2004; Xu et al. 2008) are available about association of mastitis with allelic variants of *CXCR2* genes. However, *CXCR2* gene still needs to be explored for SNPs associated with mastitis resistance in different cattle population. In the present study, we tried to identify the SNPs in *CXCR2* receptor gene of cattle, using polymerase chain reaction–single-strand conformation polymorphism (PCR–SSCP) followed by sequencing. Polymorphism in *CXCR2* gene was analysed for its association with the mastitis phenotype in cattle.

Material and methods

Experimental animals

The present study was conducted on Vrindavani crossbred cow, a crossbred synthetic strain of cattle (a cross of Holstein Friesian, Jersey, Brown-Swiss and Hariana), maintained at cattle and buffalo farm, Indian Veterinary Research Institute, Izatnagar. A total of 95 crossbred cows (42 healthy animals and 53 clinical mastitis group) were selected for this study. Cows which had never been affected by clinical mastitis during their productive life and tested negative for California Mastitis Test (CMT) were kept in the mastitis tolerant group. Whereas, cows affected with clinical mastitis at least once during their productive life were kept in the clinical mastitis group. CMT was conducted during milking of cows as per method described by Schalm and Noorlander (1957). About 10 ml of venous blood was collected from each animal under sterile conditions from jugular vein in vacutainer containing EDTA. Genomic DNA was isolated from the blood samples following standard phenol-chloroform extraction protocol (Sambrook & Russell 2001). The purity of genomic DNA was assessed spectrophotometrically.

*Corresponding author. Email: maheshdige@gmail.com

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Polymerase chain reaction

Amplification of two fragments (209 and 286 bp) of the exon 2 of *CXCR2* gene was done using two sets of primers. Fragment-1 of 209 bp (179–387 bp of exon 2) was amplified using forward primer 5′-TCTTC TTGCTAAGCCTCCTGGGAA-3′ and reverse primer 5′-TTCCTTCAGGAGTGAGACCACCTT-3′, whereas, the fragment-2 of 286 bp (804–1089 bp of exon 2) was amplified using forward primer 5′-CTACAACCTGGTCCTGATCGTG-3′ and reverse primer 5′-TCAGAGGGTAGTAGACGTGTTC-3′. The 25 μl of PCR reaction mixture was prepared using 10 pmoles of each primer, 2.5 mM of dNTPs, 1.5 mM MgCl2, 1 × PCR buffer, 80–100 ng DNA template and 1 U Taq DNA Polymerase. The optimisation of thermal cycle conditions was done as follows: 4 min at 94°C; followed by 30 cycles of 94°C for 1 min, 63°C for 30 sec for 209 bp, 62.2°C for 286 bp fragment, 72°C for 30 sec; finally final extension of 10 min at 72°C.

SSCP analysis

SNPs were screened in these fragments using SSCP technique (Orita et al. 1989). The 209 and 286 bp PCR products were resolved on 12 and 15% polyacrylamide gel, respectively. For visualisation of bands, silver staining was carried out as per the method described by Bassam et al. (1991).

DNA sequencing and sequence analysis

After the polymorphism was detected, the purified PCR products of different electrophoresis patterns were sequenced in both directions, and these sequences were analysed with the DNA star software. These sequences were submitted online in NCBI gene bank with accession number (GeneBank Accession No. JF 927834 and JF 927835).

Statistical analysis

The PROC LOGISTIC of SAS 9.2 was used for statistical analysis with model given below:

$$Ln(P_i/1-P_i) = \beta_0 + \beta_A X_A + \beta_G X_G + \beta_Y X_Y + \beta_S X_S + \varepsilon$$

where P_i = probability that animal i was mastitic (1 = mastitis; 0 = no mastitis); β_0 = the intercept; β_A = the regression coefficient for parity; β_G = the regression coefficient for season; β_Y = the regression coefficient for amplicon 1; β_S = the regression coefficient for amplicon 2; X_A, X_G, X_Y, X_S = the dummy variables for presentation of effects of parity, season, amplicon 1 and amplicon 2; and ε = random error term.

Results and discussion

SSCP analysis

SSCP patterns were detected for 209 and 286 bp fragments. The frequency of different genotypes of animals revealed from both fragments is being tabulated in Table 1. The two patterns of SSCP for amplicon 1 (209 bp) revealed genotypes AA and AB (Figure 1). The BB genotype pattern could not be found in the present investigation. Three different patterns of amplicon 2 (286 bp) of *CXCR2* exon 2 was recorded as AA, AB and BB (Figure 1). In order to better understand the detailed genetic variations of the *CXCR2* gene, all polymorphic SSCP patterns were sequenced. These sequences were submitted to NCBI GenBank data (Accession no. JF927834 and JF927835). Comparison between the nucleotide sequences of the cattle *CXCR2* gene (GeneBank Accession No. NC_007300) and the sequencing result revealed four novel SNPs at A214G, T340C, G344A, C856T and G1027A. The sequence alignment reports of both fragments are presented in Figures 2 and 3, respectively.

Association study

One of the important objectives of the current study was to find an association of variation in *CXCR2* gene with the mastitis phenotype in the Vrindavani cattle. The association of the mastitis phenotype with genotypes could not be established although; the polymorphic patterns for the *CXCR2* gene were detected. The model could estimate the effect of several genetic and non-genetic factors on the occur-

Table 1. Genotype frequency for 209 and 286 bp in exon 2 of *CXCR2* gene.

Fragment	Genotype	Healthy animals	Clinical mastitis
Amplicon 1 (209 bp)	AA	0.48	0.58
	AB	0.52	0.42
Amplicon 2 (286 bp)	AA	0.05	0.04
	AB	0.36	0.38
	BB	0.59	0.58

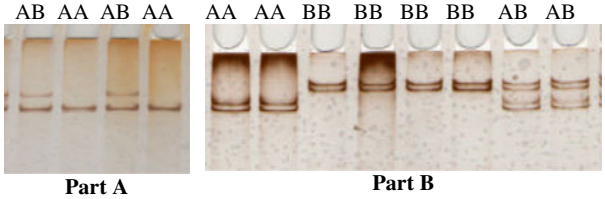

Figure 1. PCR–SSCP patterns of 209 bp (Part A) and 286 bp (Part B) of exon 2 of the *CXCR2* gene.

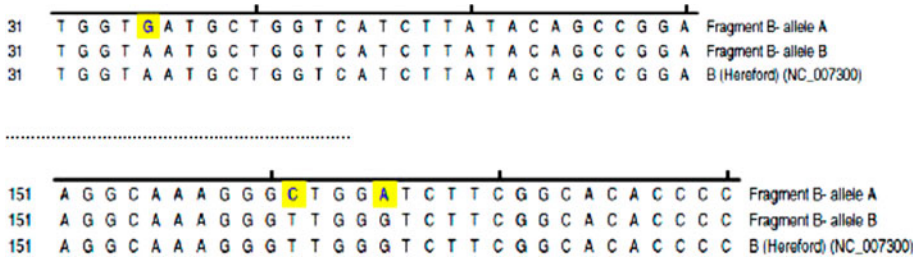

Figure 2. Nucleotide sequence alignment of amplicon 1 (209 bp) of *CXCR2* gene of Vrindavani crossbred cattle with Hereford cattle.

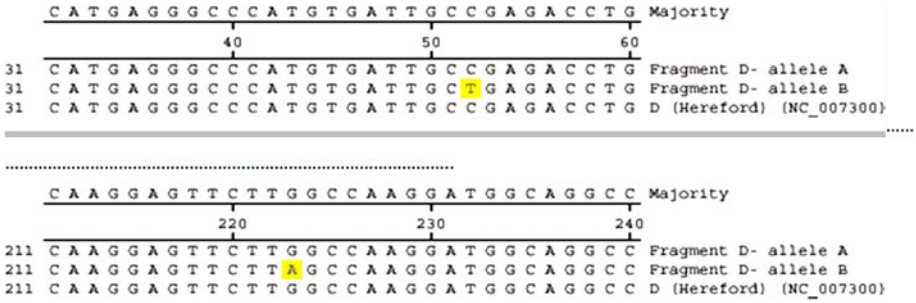

Figure 3. Nucleotide sequence alignment of amplicon 2 (286 bp) of *CXCR2* gene of Vrindavani crossbred cattle with Hereford cattle.

rence of mastitis. From the analysis, it was found that only parity had a significant effect on the occurrence of mastitis ($P = 0.03$). All the other factors had non-significant effect on the occurrence of mastitis (Table 2). In the present investigation, it was revealed that the different genotypes of *CXCR2* were not significantly associated with occurrence of mastitis. Genetic polymorphism of investigated fragments of *CXCR2* (exon 2) did not show significant association with the occurrence of mastitis. On the contrary, Xu et al. (2008) reported five alleles of *CXCR2* gene, based on the three SNPs (684, 777 and 861 bp), where three genotypes were associated with the incidence of mastitis. SNPs located within other regions of the bovine *CXCR2* gene may also affect gene function during mastitis infections (Youngerman et al. 2004). In cattle, other non-synonymous SNPs located within segments of the *CXCR2* coding sequence may also affect receptor binding and functional activities. Additionally, either synonymous or non-synonymous SNP in the promoter and 3′ untranslated regions may also affect functional activities of the gene. The results of this study demonstrated that *CXCR2* locus in the Vrindavani cattle is polymorphic. Moreover, these findings did not support the association of *CXCR2* alleles with resistance and susceptibility of Vrindavani cattle to clinical mastitis.

Table 2. Effect of various factors on incidence of mastitis in Vrindavani cattle.

Type III: Analysis of effects			
Effect	DF	χ^2	$P > \chi^2$
Parity	4	10.462	0.033
Season	3	1.794	0.616
Amplicon 1 (209 bp)	1	0.264	0.607
Amplicon 2 (286 bp)	2	1.410	0.494

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