

Molecular Characterization of TLR2 Gene Exons and Its Association with SCS in HF Crossbred Cattle



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ABSTRACT

Present study was carried out to investigate genetic polymorphism of TLR2 gene exon 1 and exon 2 in 214 HF crossbred cow. Molecular characterization of TLR2 gene was carried out by PCR- SSCP analysis. The study revealed presence of six SNPs viz., A827G, C1088G, C2155A, G2281C, G2410A and C2600T in exon 2 of TLR2 gene. SNP G2410A and C2600T were found to be significantly associated with SCS ($p < 0.1+$). It was found that for SNP G2410A, genotype GG (LSM for SCS 3.089 ± 0.197) showed lower SCS than the genotype AA (LSM for SCS 3.375 ± 0.176) and AG (LSM for SCS 3.447 ± 0.197). Similarly for SNP C2600T, genotype CC (LSM 3.089 ± 0.197) showed moderately significantly lower SCS ($p < 0.10+$) than the genotype TT (LSM 3.375 ± 0.176) and CT (LSM for SCS 3.447 ± 0.197). In conclusion, it is suggested that TLR2 gene could be a candidate gene associate with resistance to mastitis for molecular marker-assisted selection and breeding.

INTRODUCTION

Mastitis is the most common problem faced by the dairy producers all over the world. Recovery from inflammation due mastitis depends on the host defense mechanism. Toll like receptors (TLRs) are one of the most recently identified molecules found to play the pivotal role in innate as well as acquired immunity (Akira *et al.*, 2001). They are cell-surface receptors which recognize a broad class of pathogen-associated molecular patterns (PAMPs) that activates innate and adaptive immune responses and confer disease resistance (Shizuo *et al.*, 2001). Among all the 13 bovine TLRs, TLR2 has its own specific role for stimulation and activation of immunity against infection. Present study was conducted to study molecular character of TLR2 gene and associating the polymorphism with (Somatic Cell Score) SCS in HF crossbred cattle.

MATERIALS AND METHODS

Animals

A total of 214 animals were used in this study. Blood samples were collected aseptically in vacutainer tubes containing 0.5% EDTA and stored at 4°C. DNA isolation was performed by using a modified high salt method as described by Miller *et al.* (1988) as soon as possible, the delay not exceeding 24 hrs. Milk sample was collected from each lactating animal to estimate the somatic cell count (SCC) using microscopic examination method (Singh and Ludri, 2000) and digital somatic cell counter.

Primers and PCR Amplification

Primers reported by Zhang *et al.*, 2009 for exon 1 and exon 2 of TLR2 gene were used to amplify the region of interest. The polymerase chain reactions (PCR) were carried out in a total volume of 25 µl solution containing 100 ng template DNA, 1X buffer (Tris-HCl 100 mmol/l, pH 8.3; KCl 500 mmol/l), 0.5 mmol/l primers, 2.0 mmol/l MgCl₂, 0.25 mmol/l dNTPs, and 0.5U Taq DNA polymerase (Amnion Biosciences, Bangalore, India). PCR products were separated by electrophoresis on 1.5% agarose gels.

PCR-SSCP Analysis

PCR Single-Strand Conformation Polymorphism (PCR-SSCP) was used to detect SNPs in TLR2 gene. Aliquots of 10 µl PCR products were mixed with 10 µl denaturing solution (95% formamide, 25mM EDTA, 0.025% xylene cyanole, and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice for 5 min. The samples were separated by an electrophoresis on a 10% neutral polyacrylamide gel (acrylamide:bisacrylamide= 29:1) at

140 volt for 10–12 hours. The gels were stained with Ethidium Bromide (10 mg/ml) to identify SSCP patterns.

Sequence Analysis

Representative PCR products of each electrophoresis patterns were sequenced by Xcleris Labs Ltd., Ahmadabad. Chromatogram drawn by data collection software was used to extract the sequence data. BLAST analysis was performed to confirm gene identity. Sequence alignment was carried out by Bioedit software.

Statistical analysis

Milk somatic cells were measured for each animal and then converted into somatic cell score (SCS) using formula:

$$SCS = \log^2 (SCC \text{ in lakhs}) + 1$$

The level of association of the SNP and the SCS was evaluated in a fixed model of least squares analysis considering various effects. Statistical model considered for the present analysis was;

$$Y_{ijklmnpq} = \mu + S_i + P_j + A_k + B_l + M_m + C_n + E_p + G_q + e_{ijklmnpq}$$

Where, Y is the SCS *i.e.*, \log_2 conversion of the SCC for the animals, μ global mean, S is the season of collection (i = summer, rainy or winter), P is parity or lactation number of the individual (j = 1, 2, 3, 4, 5, 6 or 8), A is the stage of lactation (k = I, II or III), B is the type of infection involved based on PCR identification of microorganism (l = gram positive organism detected, gram negative organism detected or mixed infection diagnosed), M is the level of test day per day milk production (m = 0 to 5 kg, >5 to 10 kg, >10 to 15 kg, >15 to 20 kg or >20 kg), C is the effect of genetic group (n = HF crossbred and graded HF), E is the method of milking practiced (p = hand milking and machine milking).

RESULTS

PCR amplification and SSCP analysis

Genomic DNA of HF crossbred cattle was successfully amplified using seven primer pairs for TLR2 gene, and the PCR products were separated by electrophoresis on 1.5% agarose gels. Different migration patterns of the single stranded DNA of same molecular weight were seen for exon 2.2, exon 2.3, exon 2.5 and exon 2.6 fragments due to presence of polymorphic sites.

Sequencing

The representative samples of each pattern were sequenced and

aligned by using Bioedit software. Sequencing revealed presence of six SNPs viz., *A827G* (exon 2.2), *C1088G* (exon 2.3), *C2155A* (exon 2.5), *G2281C* (exon 2.5), *G2410A* (exon 2.6) and *C2600T* (exon 2.6) in exon 2 of *TLR2* gene.

Genotype frequencies and allelic frequencies of *TLR2* gene:

Genotype and allele frequencies of *TLR2* gene in 214 HF crossbred cattle were presented in Table 1. It was seen that the SNPs identified in the present study has relatively low genotype frequencies for all the loci.

Table 1. Genotype and allele frequencies of *TLR2* SNPs Locus

Locus	Genotype	Genotype frequency	Allele frequency
A827G	AA	0.66	0.6776
	AG	0.04	
	GG	0.30	0.3224
C1088G	CC	0.74	0.2523
	CG	0.02	
	GG	0.24	0.0187
C2155A	CC	0.58	0.7196
	CA	0.27	
	AA	0.15	0.2804
G2281C	GG	0.15	0.2804
	GC	0.27	
	CC	0.58	0.7196
G2410A	GG	0.26	0.3692
	GA	0.22	
	AA	0.52	0.6308
C2600T	CC	0.26	0.3692
	CT	0.22	
	TT	0.52	0.6308

Association of the *TLR2* gene polymorphisms with SCS

The relationships between polymorphisms and SCS were evaluated. SNPs *G2410A* and *C2600T* were significantly associated with SCS ($p < 0.10$). For SNP *G2410A*, when a fixed model for least square analysis was used genotype GG showed lower SCS than the genotype AA and AG. Similarly, for SNP *C2600T*, the genotypes CC showed significantly lower SCS ($p < 0.10$) than the genotype TT. The least squares mean and standard error for SCS of SNP *G2410A* and *C2600T* genotypes in dairy cattle were given in Table 2.

Table 2. Association of SNP *G2410A* and *C2600T* with SCS in HF crossbred cattle SNP Genotype LSM \pm SE and p value

SNP	Genotype	LSM \pm SE	p value
G2410A	AA	3.375 \pm 0.176	0.063*
	AG	3.447 \pm 0.197	
	GG	3.089 \pm 0.197	
C2600T	CC	3.086 \pm 0.136	0.063*
	CT	3.465 \pm 0.145	
	TT	3.375 \pm 0.115	

DISCUSSION

TLR2 is a membrane protein and plays a role as a specific receptor which is expressed on the surface of cells and recognizes *PAMPs* associated with a variety of microorganisms. Six SNPs were identified in the study and the SNPs identified were located in the coding region of bovine *TLR2* gene and resulted in presumed amino acid changes in *TLR2* protein. Perusal of literature suggested association of *TLR2* gene polymorphism with mammary health [Zang *et al.*, 2009; Pant *et al.*, 2007; Mariotti *et*

al., 2009; Hung *et al.*, 2011; Bai *et al.*, 2011 and Bai *et al.*, 2012].

With a background to associate the genetic polymorphism in *TLR2* gene with mastitis, present study was directed towards association of the SNPs with SCS. SNPs *G2410A* and *C2600T* were significantly associated with SCS. Genotype GG showed lower SCS than the genotype AA and AG also genotypes CC showed lower SCS than the genotype TT and CT. The SNPs described in the present study were all unique SNPs and have not been reported till date. Present study however appends to the results of the previous researchers [Zang *et al.*, 2009; Pant *et al.*, 2007; Mariotti *et al.*, 2009; Hung *et al.*, 2011; Bai *et al.*, 2011 and Bai *et al.*, 2012] and provides a background that *TLR2* gene could be a candidate gene associate with occurrence of mastitis for molecular marker-assisted selection breeding.

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