Veterinary World, EISSN: 2231-0916
Available at www.veterinaryworld.org/Vol.7/December-2014/2.pdf

RESEARCH ARTICLE
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Cluster of differentiation 14 gene polymorphism and its association with incidence of clinical mastitis in Karan fries cattle

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Received: 06-08-2014, **Revised:** 04-11-2014, **Accepted:** 07-11-2014, **Published Online:** 04-12-2014

doi: 10.14202/vetworld.2014.1037-1040. **How to cite this article:** Selvan AS, Gupta ID, Verma A, Chaudhari MV, Kumar V (2014) Cluster of differentiation 14 gene polymorphism and its association with incidence of clinical mastitis in Karan fries cattle, *Veterinary World* 7(12): 1037-1040.

Abstract

Aim: The present study was undertaken with the objectives to characterize, identify DNA polymorphism in cluster of differentiation 14 (CD14) gene in Karan Fries (KF) cattle and to analyze association between genetic variants with incidence of clinical mastitis in National Dairy Research Institute (NDRI) herd, Karnal.

Materials and Methods: Genomic DNA was extracted using blood of randomly selected hundred KF lactating cattle by phenol-chloroform method. After checking its quality and quantity, polymerase chain reaction (PCR) was carried out using reported primers to amplify 832 base pair region covering nucleotide base position number 1012 to 1843 (part of promoter, 5'UTR, exon 1, intron 1 and part of exon 2) of bovine CD14 gene. The PCR amplified target product was purified, sequenced and further ClustalW analysis was done to align edited sequence with reported *Bos taurus* sequence (EU148610.1). The restriction fragment length polymorphism (RFLP) analysis was performed for each KF cow using *Hinf*I restriction enzyme (RE). Cows were assigned genotypes obtained by PCR-RFLP analysis and association study was done using Chi-square (χ^2) test.

Results: After PCR amplification, DNA sequencing of amplicon confirmed the 832 bases covering 1012 to 1843 nucleotide base position of bovine CD14 gene. ClustalW multiple sequence alignment program for DNA revealed six nucleotide changes in KF cows at positions T1117D, T1239G, T1291C, G1359C, G1361A, and G1811A. Cows were also screened using PCR-RFLP with *Hinf*I RE, which revealed three genotypes CC, CD and DD that differed significantly regarding mastitis incidence. Within CC genotype, 72.73% of cows were in a mastitis non-affected group whereas, those in CD and DD genotypes 69.44% and 60.38% respectively were mastitis affected.

Conclusion: KF cows with allele C of CD14 gene were less susceptibility to mastitis compared with D allele.

Keywords: cluster of differentiation 14, *Hinf*1, Karan Fries, mastitis, restriction fragment length polymorphism, single nucleotide polymorphism.

Introduction

In India, crossbred cattle are gaining much importance being high milk producer compared to indigenous cattle, which could be observed by recent figures of county's milk production revealing that out of total cattle milk production more than half is contributed by crossbred cattle [1]. Karan Fries (KF) was developed by crossing Holstein Friesian bulls with Tharparkar cows at National Dairy Research Institute (NDRI) (Karnal) [2]. However, the major drawback of crossbred cows is their poor adaptability and susceptibility to infectious diseases especially mastitis when compared with indigenous cattle.

Genes play one of the major roles in disease resistance in bovine species [3-10]. Candidate genes such as BoLA-DRB3 [11, 12], FEZ [13], TLR4 [14, 15], CARD 15 [16, 17], cluster of differentiation 14 (CD14) [18-20] and many more genes are associated with mastitis resistance/susceptibility in different cattle breeds. CD14 gene is one of the excellent candidates for mastitis resistance in cattle and has been mapped on BTA 7 [21]. Size of CD14 gene is 2630 bp comprising of 2 exons and 1 intron. The total coding sequence of CD14 gene is 1122 bp that encodes 373 amino acids [18].

Since there is no report on CD14 gene polymorphism in KF cattle the present study was carried out with the objective to characterize and identify genetic polymorphism in CD14 gene and to explore association of this region with incidence of clinical mastitis in KF cattle.

Materials and Methods

Ethical approval

The experiment was approved by Institutional animal ethics committee

Blood samples of one hundred KF lactating cattle maintained at Livestock Research Centre of NDRI (Karnal) were collected from the cattle with history of incidences of clinical mastitis (affected ≥ once) and also the non-affected. Genomic DNA was extracted by phenol-chloroform method as described by Sambrook and Russel [22] with minor

modifications. Quality of genomic DNA was checked on 0.6% agarose gel electrophoresis while its quantification was done using Nanodrop spectrophotometer method. Polymerase chain reaction (PCR) was carried out using CTTCCTGTTATAGCCCCTTTCC and CACGATACGTTACGGAGACTGA as forward and reverses gene-specific oligonucleotide primers, respectively, as reported by Ibeagha-Awemu *et al.* [18] to amplify 832 base pair region covering nucleotide base position number 1012-1843 (part of promoter, 5'UTR, exon 1, intron 1 and part of exon 2) of bovine CD14 gene. The PCR reaction mixture was incubated in thermal cycler initially at 94°C for 2 min followed by 34 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 40 s, and a final extension of 72°C for 10 min. The confirmation of each PCR amplification of desired target was done using 2% agarose gel electrophoresis. The PCR amplified target product was purified and sequenced by outsourcing (M/s. SciGenom Labs Pvt., Ltd.). The raw sequence was edited (BioEdit) and further ClustalW analysis was done to align edited sequence with reported *B. taurus* sequence (EU148610.1). The restriction fragment length polymorphism (RFLP) analysis was performed for each KF cow using *Hinf*I restriction enzyme (RE). PCR products of each animal were digested with *Hinf*I RE (0.4 ml) at 37°C for 16 h. Fragments of RE digestion were separated on 2.5% agarose gel and photographed using gel documentation system. Cows were grouped as mastitis affected and not affected and were assigned genotypes obtained by PCR-RFLP analysis. Association study was done using the Chi-square (χ^2) test.

Results and Discussion

Genomic DNA was extracted from blood of 100 KF cows. Targeted 832 bp region (part of promoter, 5'UTR, exon 1, intron 1 and part of exon 2) of bovine CD14 gene from each DNA sample was amplified using specific primer pairs in thermal cycler (Figure-1).

DNA sequencing of the amplicon confirmed the 832 bases covering 1012-1843 nucleotide base position of bovine CD14 gene. Further, these nucleotide sequences of KF cows were compared with that of *B. taurus* (EU148610.1) using ClustalW multiple sequence alignment program for DNA and six nucleotide changes in KF cows at positions T1117D, T1239G, T1291C, G1359C, G1361A and G1811A were observed. Out of these six single nucleotide polymorphisms (SNPs) three were for thiamine and three for guanine (Figures-2 and 3). All SNPs except at position 1117 base pair were similar to that observed by Kumar *et al*. [20] in Sahiwal (*Bos indicus*) cows.

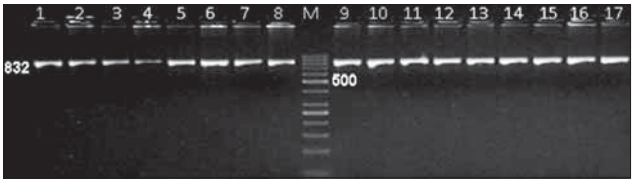


Figure-1: Polymerase chain reaction (PCR) product of 832 bp target region of cluster of differentiation 14 gene in Karan fries cows

Association analysis of RFLP fragments of CD14 gene-targeted region with clinical mastitis

*Hinf*I RE digestion of 832 bp PCR amplified product of KF CD14 gene exhibited five fragments of 47, 183, 225, 272 and 377 bp, resolving into three genotypes. Cows with band patterns 377, 272 and 183 bp were assigned genotype CC while those with 377, 272, 225, 183 and 47 bp were assigned genotype CD and 377, 225, 183 and 47 bp as genotype DD (Figure-4). The DD genotype was highest followed by CD and CC with 0.53, 0.36 and 0.11 genotypic frequencies respectively in the studied population. The frequency of D allele was 0.71 and that of C was 0.29. However, the findings are different from that of Kumar et. al. [20] where, he reported genotype CC and allele C with highest frequency for the same targeted region of 832 bp of CD14 gene using *Hinf*I RE in Sahiwal cows.

Chi-square (χ^2) analysis revealed that all three genotypes of KF cattle differ significantly regarding mastitis incidence. Within CC genotype, only 27.27%

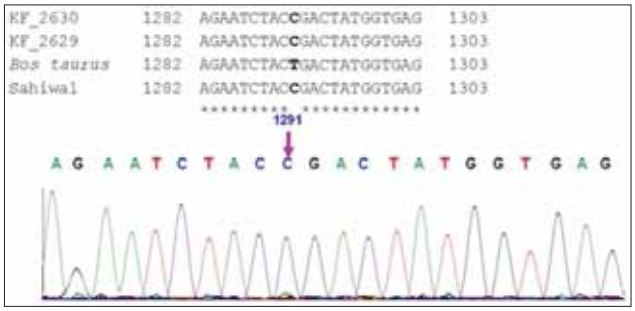
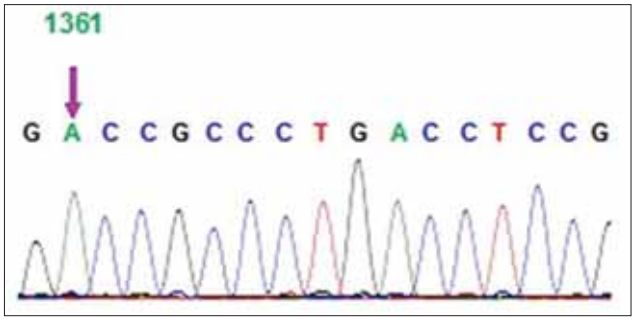


Figure-2: Chromatogram showing nucleotide change at position 1291(T>C)



KF_2630	1360	GACCGCCCTGACCTCCG	1376
KF_2629	1360	GACCGCCCTGACCTCCG	1375
Bos taurus	1360	GGCCGCCCTGACCTCCG	1376
Sahiwal	1360	GGCCGCCCTGACCTCCG	1376
		* **********	

Figure-3: Chromatogram showing nucleotide change at position 1361(G>A).

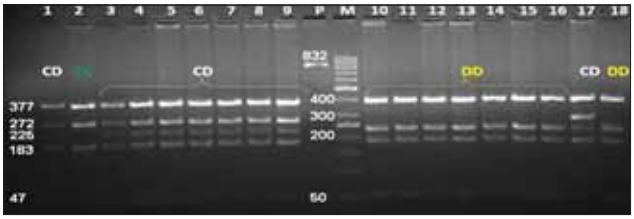


Figure-4: Polymerase chain reaction (PCR) restriction fragment length polymorphism analysis of 832 bp target region of cluster of differentiation 14 (CD14) using *Hinf*I restriction enzyme in Karan Fries cows. Lane 2: CC (372, 272 and 183 bp), Lane 1, 3-9, 17: CD (377, 272, 225, 183, 47 bp), Lane 10-16, 18: DD (377, 225, 183, 47 bp), P: PCR product (832 bp), M: 50 bp DNA ladder

cows were mastitis affected, whereas, 69.44% and 60.38% cows were mastitis affected within CD and DD genotypes respectively. Hence, it is inferred that allele C is desired allele of CD 14 gene with respect to a lesser incidence of mastitis in KF cows.

Conclusions

The nucleotide sequencing of the targeted region of 832 bp (part of the promoter, 5'UTR, exon 1, intron 1 and part of exon 2) of bovine CD14 gene revealed six SNPs in KF cattle breed. PCR-RFLP analysis of the same region showed three patterns with significant association with incidence of clinical mastitis.

Author's Contributions

IDG conceived and designed the work. ASS conducted experiment. ASS and MVC done analysis, association study and VK assisted in writing of the manuscript. IDG and AV helped in the revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors are thankful to Head, DCB Division for providing necessary research facilities. The authors also thankful to the Director (NDRI) for financial assistance provided during the research work.

Competing Interests

The authors declare that they have no competing interest.

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