Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(4): 1112-1117

Received: 03-01-2020
Accepted: 04-02-2020

Rajesh Kumar S
Dairy Cattle Breeding Division, National Dairy Research Institute, Karnal, Haryana, India

Gupta ID
Dairy Cattle Breeding Division, National Dairy Research Institute, Karnal, Haryana, India

Raja KN
National Bureau of Animal Genetic Resources, Karnal, Haryana, India

Virendra Kumar
Livestock Research Station, Kodamdesar, Bikaner, Rajasthan, India

Saravanan R
Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Kathiravan Periasamy
Animal Production and Health Laboratory, Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna, Austria

Corresponding Author:
Rajesh Kumar S
Dairy Cattle Breeding Division, National Dairy Research Institute, Karnal, Haryana, India

Sequence characterization of S100A8 gene and its 5' flanking region in Indian Zebu *(Bos indicus)* and crossbred *(Bos indicus X Bos Taurus)* cattle

Rajesh Kumar S, Gupta ID, Raja KN, Virendra Kumar, Saravanan R and Kathiravan Periasamy

Abstract
The present study was carried out to sequence characterize bovine S100A8 gene in Sahiwal (*Bos indicus*) and Karan Fries (*Bos indicus* X *Bos taurus*) cattle. S100A8 sequence data including 912 bp of coding DNA and 1kb of 5' flanking region were generated in Sahiwal and Karan Fries cattle. The S100A8 coding DNA sequence of Sahiwal and Karan Fries cattle were found to have 100% identity with *Bos taurus* cattle, 99% with yak, 97% with buffalo and 85% with horse. A total of 18 putative sites were identified in 1kb region upstream to S100A8 gene and the location of most of these transcription factors except CP2 and NF kappa B were conserved in Sahiwal and Karan Fries cattle. The putative domain for binding CP2, found in Sahiwal cattle was absent in Karan Fries cattle while NF kappa B found in Karan Fries cattle was absent in Sahiwal.

Keywords: S100 A8, phylogeny, coding DNA, transcription factor

Introduction
Mastitis is one of the most common and economically important diseases in cattle [1, 2]. Breeding cattle with enhanced host resistance against mastitis is one of the long-term strategies to improve udder health and help reducing the financial losses to farmers. Several candidate genes have been reported to significantly influence the host resistance/susceptibility to mastitis in cattle [3, 4]. S100 proteins, one such candidate, constitute the largest gene family within the EF-hand protein super family. In 1965 Moore isolated the first protein members of the S100 family, S100A1 and S100B from bovine brain. Many other members of this family were identified in the following years based on sequence homology and similar structural properties [5]. S100 proteins are small acidic proteins (9-14 kDa) and contain two distinct EF-hand motifs. C terminal EF–hand contains a classical Ca^{2+} binding motif. These proteins contribute to key cellular pathways involving calcium as a second messenger.
S100A8 (calgranulin A) is a member of the S100 family of Ca^{2+} binding proteins. S100A8 and S100A9 together form a heterodimer called calprotectin which has antimicrobial properties and plays an important role in innate immunity. S100A8 can be produced by a variety of cell types including neutrophils, activated macrophages, keratinocytes and fibroblasts and their presence acts as a marker for inflammation in different tissues [6]. S100A8 has been reported to be differentially expressed with respect to mastitis in Holstein cattle [3]. Till date, little or no report is available on the structure and polymorphism of S100A8 genes in Indian cattle, particularly the indigenous Zebu *(Bos indicus)* cattle. Hence, the present study was undertaken with the following objectives: (i) to characterize S100A8 gene and its promoter in Sahiwal (*Bos indicus*) and Karan Fries (*Bos indicus X Bos taurus*) cattle and (ii) to identify putative transcription factor binding sites in the 5' flanking region that could potentially alter S100A8 expression in Indian cattle.

Materials and Method
Sampling and DNA extraction
Blood samples were collected from one representative Sahiwal and Karan Fries cattle maintained at Cattle Farm, National Dairy Research Institute (NDRI), Karnal. About 10 ml of blood was collected in a sterile 0.5% EDTA (10 µl/ml of blood) coated vacutainers by jugular venipuncture method.

The collected blood was stored in the refrigerator at 4 ˚C till further processing for extraction of DNA. DNA was isolated from blood using standard phenol-chloroform method as described in Sambrook and Russell (2001) [7] with minor modifications. Quality and quantity of extracted DNA was determined by using Nanodrop spectrophotometer.

Primer designing, PCR amplification and sequencing

Sequence characterization of bovine S100A8 gene was carried out by primer walking method. This method works by PCR amplification of whole gene in fragments that overlap with each other at 5' and 3' ends. S100A8 gene was sequence characterized by synthesizing five overlapping fragments to generate the complete sequence of about 2.05 kb covering all exons and 5' upstream regulatory and flanking regions. Five sets of oligonucleotide primers reported by Kathiravan *et al*. (2011) [8] was utilized for generating overlapping PCR fragments of complete S100A8 gene. Polymerase chain reaction was carried out in a final reaction volume of 20 µL, containing ~50ng of genomic DNA, 5 pmole of each primer, 200 µM of each dNTP, 2 µL of 10X buffer with 1.5 mM MgCl2 and one unit of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore India). Amplification was performed using programmable thermal cycler (PTC-200, MJ Research, USA) with an initial denaturation at 95 ˚C for 2.5 min followed by 35 cycles of 94 ˚C for 30s, respective annealing temperature for 30s and 72 ˚C for 1min, with a final extension for 5 min at 72 ˚C. The amplified PCR products were checked by running on 2% agarose gels in 1X TAE along with suitable standard size DNA marker. The amplified products were visualized, size estimated and documented using gel documentation system. The amplicons from each of the overlapping fragments were subjected to custom DNA sequencing from both ends (5' and 3' ends) using forward and reverse primers (M/S Chromous Biotech, India).

Sequence data analysis

Raw sequence data were edited using Chromas (Ver. 1.45, http://www.technelysium.com. au/chromas.html). The edited sequences were saved in EditSeq module of LASERGENE software (DNASTAR Inc, Madison, WI, USA) for subsequent analysis. The forward and reverse sequences from each of the fragments from S100A8 gene were assembled together manually after multiple alignment with *Bos taurus* sequence template. Multiple alignments of different sequences were performed using Clustal W program of BIOEDIT and CLC FreeWorkbench. Sequences from different overlapping fragments were further assembled to derive the contiguous and complete sequence of S100A8 gene. The structure of *Bos indicus* S100A8 gene including its exon-intron junctions were predicted using GENSCAN web server available at http://genes.mit.edu/GENSCAN.html [9].

The sequence homology among the coding DNA sequences of S100A8 gene belonging to different species was estimated by Basic Local Alignment Search Tool Analysis (BLAST) at National Centre for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic analysis of S100A8 gene sequences from various species was performed using CLC Free Workbench. Unweighted paired group method with arithmetic mean (UPGMA) algorithm was followed to derive the dendrogram with bootstrap re-sampling of 1000 subsets of sequence data under analysis. Various binding sites for putative transcription factors in the 5' flanking region were identified using TESS (Transcription Element Search Software) and MATCH [10]. The transcription factor binding sites were predicted from the publicly available database of sequence matrices corresponding to different transcription factors. The option to minimize both error rates in predicting transcription factor binding sites were used for MATCH analysis. The factors with scores below 1.0 for core match and 0.9 for matrix match were not considered for subsequent analysis.

Results and Discussion

In the present study, the complete S100A8 gene sequences of Sahiwal and Karan Fries cattle were generated with a total contig length of 2.036 kb and 2.080 kb respectively. This also included ~1 kb of 5' flanking region covering the basal promoter complex of S100A8 gene. The complete S100A8 gene sequences generated in the present study were submitted to NCBI-GenBank and are available at accession numbers JX070095 and JX070094.

Structure of S100A8 gene

The coding DNA sequence of S100A8 gene in cattle is 270 bases which produces a chain of polypeptide with 89 amino acids. The schematic illustration of structure and genomic organization of S100A8 gene in cattle is presented in Figure 1 and the complete annotated sequence of the gene along with 5' upstream promoter and flanking sequence in *Bos indicus* cattle is presented in Figure 2. The structure of S100A8 gene included three exons separated by two introns. The first exon of 36 base pairs codes for 5' untranslated sequences, the second exon partially codes for 5' untranslated sequences (first 20 base pairs) and amino-terminal EF hand (141 base pairs) while the third exon codes for the carboxy-terminal EF hand. The length of third exon was 129 base pairs. The length of first intron was 0.434 kb and 0.436 kb in Sahiwal and Karan Fries cattle respectively while the length of intron 2 was 0.150 kb in both Sahiwal and Karan Fries cattle. This finding is consistent with that of buffalo, yak, murines and humans [7].

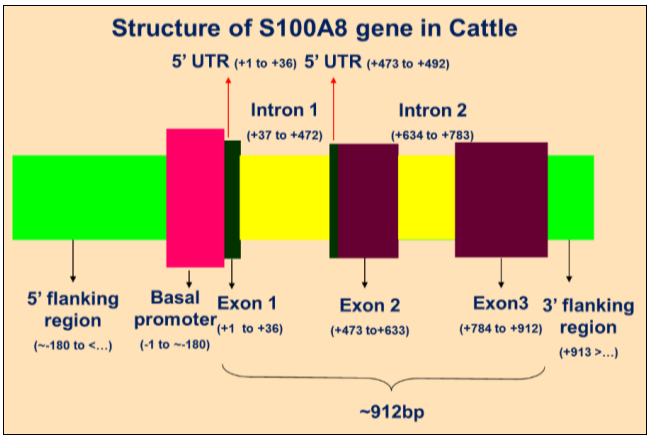

Fig 1: Schematic representation of structure of S100A8 gene in cattle

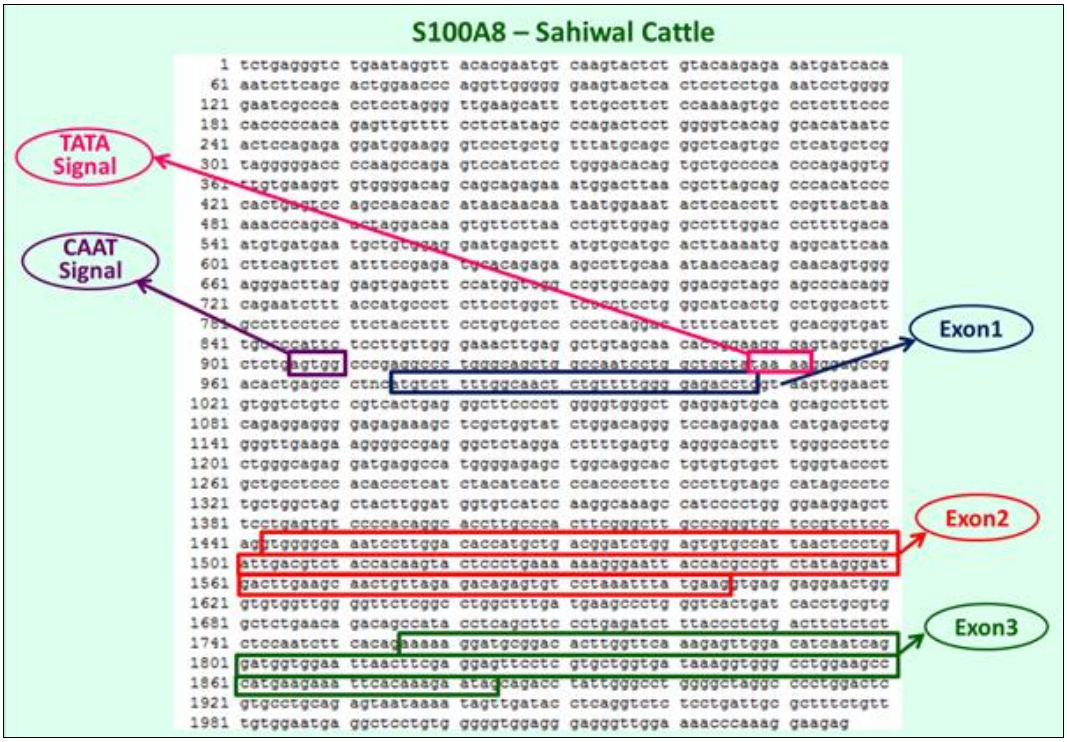

Fig 2: Complete annotated sequence of S100A8 gene along with 5' upstream promoter and flanking sequence in *Bos indicus* (Sahiwal) cattle (GenBank Accession Number - JX070095)

Sequence variation of S100 A8 gene among different bovine species

The details of sequence variation among different bovine species *viz*. *Bos taurus*, *Bos indicus*, *Bos taurus X Bos indicus* and *Bos grunniens* are presented in Table 2. Comparison of S100A8 gene sequence of different bovine species and sub-species revealed a total of eight nucleotide changes within different exonic and intronic regions. These eight nucleotide changes were observed at positions 289, 355, 356, 401, 598, 643, 713 and 884 bases from the start codon (Table 2). Among these eight nucleotide variations, six were observed in intronic regions (four within intron 1 and two within intron 2) and two in exonic regions (one each from exon 2and 3). Three out of these eight variations were found among the bovine sub-species (*Bos indicus* and *Bos taurus*) and their crossbreds (*Bos indicus X Bos taurus*). Interestingly all the three variations were due to *Bos indicus* sequence within intron1 region which included two Thymidine deletions (at positions 355 and 356 from start codon) and one Cytidine-Thymidine transition mutation. Among the nucleotide changes within exonic region of S100A8 gene, both mutations within exon 2 and 3 were found to be transversions. Both these variations among bovines were specific to yak and no nucleotide change was observed among Sahiwal and Karan Fries cattle. Consequently, conceptualized amino acid sequence of S100A8 gene was found to be very conserved among indicine cattle (Sahiwal), taurine cattle and their crossbreds (Karan Fries).

Table 1: Nucleotide variation within S100A8 gene among different bovine species/breeds

Position from start codon	Location	*Bos taurus* (BC111678)	Karan Fries	Sahiwal	Yak (HM769859)
289	Intron 1	C	C	T	C
355		T	T	DEL	T
356		T	T	DEL	T
401		A	A	A	T
598	Exon 2	C	C	C	A
643	Intron 2	A	A	A	G
713		T	T	T	C
884	Exon 3	A	A	A	T

Sequence homology and phylogenetic analysis of S100A8 gene

To compare the coding DNA sequence of S100A8 gene in cattle with that of other species and to establish their phylogenetic relationship, basic local alignment search tool analysis was performed. The S100A8 coding DNA sequence of Sahiwal and Karan Fries cattle were found to have 100% identity with *Bos taurus* cattle, 99% identity with yak, 97 with both riverine and swamp buffalo and 85% with horse. The coding DNA sequences of S100A8 gene of all available species were utilized to establish the phylogenetic relationship of cattle S100A8 gene with other species. The UPGMA (unweighted paired group method with arithmetic mean) tree showed four major distinct clusters of S100A8 gene (Figures 3). The first cluster consisted of domestic livestock with two sub-clusters formed by ruminants (bovines and bubalines) and non-ruminants (pig, dog, horse).

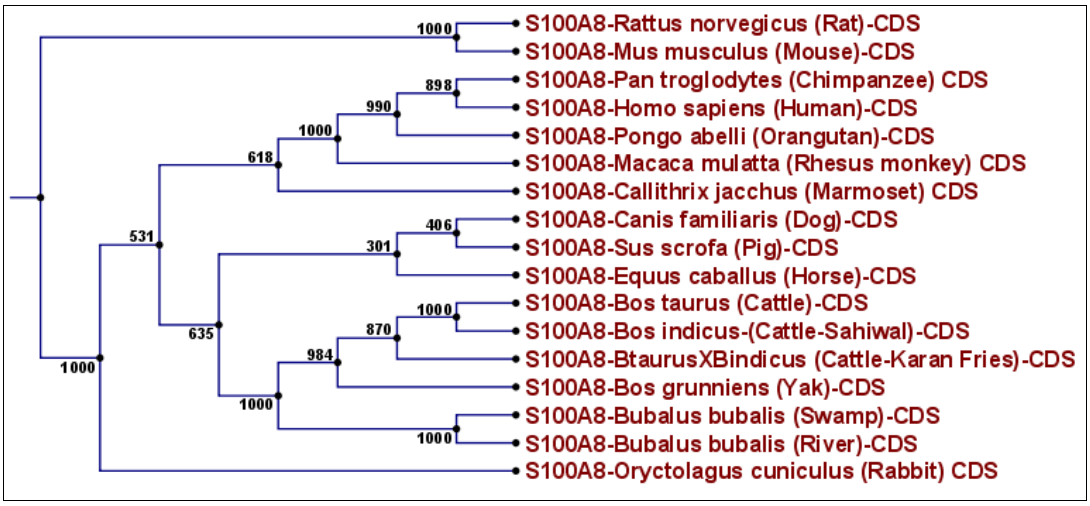

Fig 3: Phylogenetic analysis (UPGMA tree) of coding DNA sequences of S100A8 gene of different species

All the cattle sequences clustered together distinct from riverine and swamp buffalo sequences with the sequence of yak (*Bos grunniens*) placed in between. 100% bootstrap value was observed for each of the two minor clusters within this sub-cluster, one between *Bos indicus* and *Bos taurus* cattle, the other one between riverine and swamp type buffalo. This is understandable as no nucleotide variation was observed between the sub-species within these two sub-clusters. However, the nodes within non-ruminant sub-cluster had lower bootstrap values of 40.6% and 30.1%. The second major cluster of S100A8 sequences was formed by the primates which included humans, chimpanzee, orangutan, rhesus monkey and marmoset. The bootstrap values at branches within this cluster were also high ranging from 61.8% to 100%. The third major cluster was formed by rat and mouse with a bootstrap value of 99.7%. The coding DNA sequence of rabbit *(Orytolagus cuniculus)* was found to be distinct from all the other species although it was closely related to murines. The phylogenetic analysis of conceptualized amino acid sequences also revealed similar clustering with slight variations in the bootstrap values at different nodes. The findings of phylogenetic analysis in the present study broadly conformed to the report of Kathiravan *et al*. (2011) [8].

Characterization of S100A8 promoter and 5' flanking region

About 1 kb region upstream to S100A8 gene including basal promoter and 5' flanking region was sequenced in the present study. A total of 17 nucleotide changes were observed in this region among different bovine species *viz*. *Bos taurus*, *Bos indicus*, *Bos taurus X Bos indicus* and *Bos grunniens* (Table 2). Among the substitution mutations, 14 were transitions with 7 each of Adenine-Guanidine changes and Cytidine-Thymidine changes. Two mutations were found to be transversions at positions -573 (Guanidine-Cytidine) and -409 (Adenine-Cytidine) respectively.

Table 2: Nucleotide variations within 5' flanking region of S100A8 gene among different bovine species/breeds

Position	Position from first codon	*Bos taurus*	Karan Fries	Sahiwal	Yak
29	-908	G	A	A	A
48	-889	A	G	A	A
73	-864	T	T	C	C
123	-814	C	C	T	C
183	-754	A	A	A	G
226	-711	G	G	A	G
228	-709	T	T	C	C
292	-645	C	C	T	C
293	-644	A	A	G	G
302-315	-635 to -622	CAGCCTC ACAGAAC	CAGCCTC ACAGAAC	DEL	CAGCCTC ACAGAAC
364	-573	G	G	G	C
480	-457	G	G	G	A
498	-439	T	T	T	DEL
528	-409	A	A	A	C
559	-378	T	T	T	C
711	-226	C	C	C	T
931	-6	C	C	C	T
935	-2	G	G	N	G

Apart from these 17 nucleotide changes, a stretch of 14 nucleotide deletions (5'- CAGCCTCACAGAAC-3') in the region of -635 to -622 bases upstream to start codon was found in *Bos indicus* (Sahiwal) cattle. Another deletion mutation was found at position -439 upstream to start codon. Among the bovines, differences between *Bos indicus* (Sahiwal) and *Bos taurus* cattle were observed at nucleotide positions *viz*. -908, -864, -814, -711, -709, -645, -644 and at -2 bases upstream to start codon. However, *Bos taurus X Bos indicus* (Karan Fries) cattle was found to vary at only two

positions (-908 and -889) from *Bos taurus* cattle. The most visible difference within the 5' flanking region of S100A8 gene among different cattle was the 14nt deletion in Sahiwal cattle which might be specific to *Bos indicus* cattle. The nucleotide variations among different sub-species of bovines within 5' flanking region of S100A8 gene were found to be higher than reported among the sub-species of bubalines [8].

Putative transcription factor binding sites in 5' flanking region of S100A8 gene

To identify putative regulatory regions that can potentially influence expression of S100A8 gene, an *in-silico* search for possible transcription factor binding domains was performed using TESS (Transcription Element Search Software) and MATCH programs. A total of 18 different putative sites were identified in each of Sahiwal and Karan Fries cattle respectively (Table 3). All the identified sites had greater than 90% matrix similarity match (matrix similarity score=0.9) and 100% core similarity match (core similarity score=1.0). The TATA box was found between -27 to -23 bp upstream to start codon while CCAAT box was found between -69 to -65 bp upstream to the gene. The position of TATA box in cattle, buffalo and yak has been found to be consistent with that of murines and humans [11]. The putative CAAT enhancer element (C/EBP) was predicted in the region of -141 to -124 bp upstream to start codon. C/EBP proteins are a family of transcriptional factors which can induce transcription of a gene by interacting with components of basal transcription apparatus like CCAAT box. C/EBP proteins are also involved in different cellular responses like regulation of cell proliferation, growth and differentiation, metabolism and immune response. Apart from TATA box, CCAAT box and C/EBP, other predicted transcriptional factors include Zeste, Nkx2-5, STRE, RFX1, Elk-1, HNF4, HNF3β, Sox-5, CP2, v-Myb, HFH-8, NF-kappa β and Hand1/E47. The location of most of these transcription factors except CP2 and NF kappa B were conserved in the species under study. Interestingly, putative domain for binding CP2 found at position -630 to -620 in Sahiwal cattle was absent in Karan Fries cattle. This difference in the putative transcription factor binding site was due to the presence of 14 nt deletion in Sahiwal cattle. Additional nucleotides present in the Karan Fries cattle excluded the possible site for CP2 factor. Similar differences in the putative site for CP2 factor among river and swamp buffalo has been reported by Kathiravan *et al.* (2011) [8]. CP2 is a transcription factor that binds the alpha-globin promoter and activates transcription of the alpha-globin gene. It regulates erythroid gene expression, plays a role in the transcriptional switch of globin gene promoters, interacts with certain inflammatory response factors and activates many other cellular gene promoters [12]. Another finding is the putative domain for NF-kappa B found to be present at position -870 to -861 bp upstream to start codon in Karan Fries cattle was absent in Sahiwal cattle. The nucleotide change at position -864 bp upstream to start codon from Thymidine (Karan Fries) to Cytidine (Sahiwal) led to the absence of this domain in Sahiwal cattle. NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens. NF-κB plays a key role in regulating the immune response to infection (kappa light chains are critical components of immunoglobulins) [13].

Most of the putative factors identified in the present study are associated with basal, tissue-specific developmental and inducible expression of genes. Three putative sites for binding Nkx2-5 motif were predicted in both Sahiwal and Karan Fries cattle. The positions of all the three sites *viz.* -199 to -193, -395 to -389 and -478 to -472 bp upstream to start codon were conserved in both these cattle. Nkx2-5 motif binds to the homeobox protein that plays critical role in regulating tissue-specific gene expression essential for tissue differentiation as well as determining the temporal and spatial patterns of development. Two putative sites for Elk1 factor were found to be present at positions -366 to -353 and -510 to -497 in both Sahiwal and Karan

Table 3: Putative transcription factor binding sites predicted in the 5' flanking region of Sahiwal and Karan Fries cattle

Factor name	Sequence	Sahiwal		Karan Fries	
		Position from first exon	Strand	Position from first exon	Strand
NF-kappaB	GGGAAttgcc	Not Predicted		-870 to -861	+
Hand1/E47	tagcCCAGActcctgg	-768 to -753	-	-782 to -767	-
Zeste	ataatCACTCcagaga	-740 to -725	-	-754 to -739	-
HFH-8	tgcTGTTTatgca	-709 to -697	+	-723 to -711	+
Hand1/E47	caagCCAGAgtccatc	-663 to -648	-	-677 to -662	-
CP2	gccccaCCCAG	-630 to -620	+	Not Predicted	
Sox-5	acaACAATaa	-531 to -522	+	-531 to -522	+
Elk-1	caccTTCCGttact	-510 to -497	-	-510 to -497	-
v-Myb	tCCGTTact	-505 to -497	-	-505 to -497	-
Nkx2-5	acAAGTG	-478 to -472	+	-478 to -472	+
HNF-4	tggaggcCTTTGgaccctt	-459 to -441	-	-459 to -441	-
Nkx2-5	CACTTaa	-395 to -389	-	-395 to -389	-
Elk-1	ctatTTCCGagatg	-366 to -353	-	-366 to -353	-
RFX1	aaataaccacaGCAACag	-336 to -319	+	-336 to -319	+
Nkx2-5	CACTTgc	-199 to -193	-	-199 to -193	-
STRE	cCCCCTca	-166 to -159	-	-166 to -159	-
C/EBP	cggtgATTGCcccattct	-141 to -124	-	-141 to -124	-
RFX1	ttgaggctgtaGCAACac	-109 to -92	+	-109 to -92	+
Zeste	gcctctGAGTGgcccg	-76 to -61	+	-76 to -61	+

Fries cattle. Elk1 is a member of Ets family of transcription factors involved in cell proliferation and with multiple functions in brain [14]. RFX1 motif (putative site at positions -336 to -319) binds to the MHC class II regulatory factor

which is essential for its expression [15]. Hepatocyte nuclear factors (HNF4; putative site at positions -459 to -441) are a group of phylogenetically unrelated transcription factors that regulate the transcription of a diverse group of genes involved in glucose, cholesterol and fatty acid transport and metabolism.

Sox-5 is a transcription factor involved in the regulation of embryonic development [16]. Hepatocyte nuclear factor 3/fork head homolog (HFH) proteins are an extensive family of transcription factors which share homology in the winged helix DNA binding domain. Members of the winged helix family have been implicated in cell fate determination during pattern formation, in organogenesis and in cell type-specific gene expression [17]. Although, it seems unlikely that all the putative transcription factors identified in the promoter region exert their individual effects, some may actually influence the regulation of S100A8 gene in Sahiwal and Karan Fries cattle. Hence, the S100A8 promoter needs to be further assessed for its transcription factor binding capabilities and transcription efficiency.

Conclusion

In summary, the present study is the first report on sequence characterization of S100A8 gene and its 5' flanking region in *Bos indicus* cattle. A total of eight nucleotide changes within S100A8 gene and 17 nucleotide changes in its 5' flanking region was observed among different bovine species. The conceptualized amino acid sequence of S100A8 gene was conserved among indicine cattle (Sahiwal), taurine cattle and their crossbreds (Karan Fries). The study showed the presence of certain putative motifs that could potentially regulate genes involved in immune response. Elucidation of *Bos indicus* S100A8 gene variations will further form the basis to identify candidate gene markers for association with mastitis resistance/susceptibility in Indian cattle.

Acknowledgement

The authors are thankful to the Director, National Bureau of Animal Genetic Resources and Director, National Dairy Research Institute, Karnal, Haryana, India, for providing necessary facilities to the study.

References

1. Bardhan D. Estimates of economic losses due to clinical mastitis in organized dairy farms. Indian Journal of Dairy Science. 2013; 66(2):168-172.
2. Sinha R, Sinha B, Kumari R, Vineeth MR, Verma A, Gupta ID. Effect of season, stage of lactation, parity and level of milk production on incidence of clinical mastitis in Karan Fries and Sahiwal cows. Biological Rhythm Research. 2019; (In Press) doi: 10.1080/09291016.2019.1621064.
3. Ogorevc J, Kunej T, Dovč P. An integrated map of cattle candidate genes for mastitis: a step forward to new genetic markers. Acta agriculturae Slovenica. 2008; 2:85-91.
4. Ogorevc J, Kunej T, Razpet A, Dovč P. Database of cattle candidate genes and genetic markers for milk production and mastitis. Animal Genetics. 2009; 40:832-851.
5. Shang X, Cheng H and Zhou R. Chromosomal mapping, differential origin and evolution of the S100 gene family. Genetics Selection Evolution. 2008; 40:449-464.
6. Rahimi F, Hsu K, Endoh Y, Geczy CL. FGF-2, IL-1b, TGF-b regulate fibroblast expression of S100A8. FEBS Journal. 2005; 272:2811-2827.
7. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
8. Kathiravan P, Goyal S, Kataria RS, Mishra BP, Jayakumar S, Joshi BK. Sequence characterization of S100A8 gene reveals structural differences of protein and transcriptional factor binding sites in water buffalo and yak. Animal Biotechnology. 2011; 22:124-132.
9. Burge C, Karlin S. Prediction of complete gene structures in human genomic DNA. Journal of Molecular Biology. 1997; 268:78-94.
10. Kel AE, GoBling E, Reuter I, Cheremushkin E, Kel-Margoulis OV, Wingender E. MATCHTM: A tool for searching transcription factor binding sites in DNA sequences. Nucleic Acids Research. 2003; 31:3576-3579.
11. Passey RJ, Xu K, Hume DA, Geczy CL. S100A8: emerging functions and regulation. Journal of Leukocyte Biology. 1999; 66:549.
12. Kang HC, Chae JH, Lee YH, Park M-A, Shin JH, Kim SH *et al*. Erythroid Cell-Specific α-Globin Gene Regulation by the CP2 Transcription Factor Family. Molecular and Cell Biology. 2005; 25(14):6005-6020.
13. Gilmore TD. Introduction to NF-κB: players, pathways, perspectives. Oncogene. 2006; 25(51): 6680-4.
14. Besnard A, Galan-Rodriguez B, Vanhoutte P, Caboche J. Elk-1 a transcription factor with multiple facets in the brain. Frontiers in Neuroscience. 2011; 5:1-11.
15. Aftab SL, Semenec JS, Chu Chen N. Identification and characterization of novel human tissue-specific RFX transcription factors. BMC Evolutionary Biology. 2008; 8:226.
16. Wilson M, Koopman P. Matching SOX: partner proteins and co-factors of the SOX family of transcriptional regulators. Current Opinion in Genetics and Development. 2003; 12(4):441-6.
17. Peterson RS, Lim L, Ye H, Zhou H, Overdier DG, Costa RH. The winged helix transcriptional activator HFH-8 is expressed in the mesoderm of the primitive streak stage of mouse embryos and its cellular derivatives. Mechanisms of Development. 1997; 69(1-2):53-69.