PROMOTER SEQUENCE ANALYSIS OF ATP CITRATE LYASE GENE IN BUFFALO AND VECHUR CATTLE

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ABSTRACT

A study has been carried out to analyze the promoter region of ATP citrate lyase (ACLY) gene in Murrah buffalo (*Bubalusbubalis*) and native Vechur cattle. PCR amplification of the promoter region generated a fragment of 768 bp in both Buffalo and Vechur cattle.Sequence analysis revealed a few elements/consensus sequences, which have potential role in regulation of transcription. TATA box is found to be absent in the promoter region whereas, TATA like sequence, CAAT box, Cyclic AMP responsive element (CRE), Fat Specific Element (FSE), Enhancer core, Nuclear factor-k Binding site (NF-kB), E- box and inverted CAAT box are present. Comparison of these sequences between ruminants and non-ruminants revealed the presence of important sequence motifs such as, CRE, inverted CAAT boxes and a CAAT box situated far away from the transcription initiation site, together with the absence of a TATA box. All these findings correlate with down regulation of the gene resulting in a low level of expression in ruminants. Phylogenetic analysis of the nucleotide sequences revealed clustering of buffalo, Vechur cattle and Cattle on one clade and Rat and Human delineated from other species and clustered out on a separate clade.

Keywords: ACLY, promoter, sequence motifs, Murrah buffalo, Vechur cattle

INTRODUCTION

ATP citrate lyase (ACLY) is the primary enzyme responsible for the synthesis of cytosolic acetyl-CoA, the building block of lipids and cholesterol. It catalyzes the formation of acetyl-CoA and oxaloacetate from citrate and CoA with simultaneous hydrolysis of ATP (Chypre*et al.*, 2012). Lipid metabolism in ruminants differs from that of non-ruminants in a number of aspects which include microbial fermentation in the rumen and modification of dietary nutrients (Bath and Rook, 1963). Rumen microbes ferment most of the dietary carbohydrate to volatilefattyacids, acetate, propionate and butyrate, of which, acetate serves as the major lipogenic precursorin adiposetissue and mammary gland.

The activity of ACLY is reported to be low in ruminants (Ingle *et al*., 1972; Bell, 1982), which is correlated to the rate of transcription of its gene, which in turn is controlled by several factors. Promoter region of a gene plays a key role in transcriptional regulation, where several consensus sequences, destined for the attachment of factors/proteins, are present for controlling the rate of transcription. Currently, ACLY is a target for hypolipidemic intervention in humans due to

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its importance in lipogenesis and cholesterogenesis (Gribble *et al*., 1996). The development of expression systems for the recombinant human protein offered a powerful tool for research on this enzyme.

In the present study, promoter region of ACLY gene in Murrah buffalo and native Vechur cattle is PCR amplified, cloned, sequenced and analysed for the presence of various consensus sequences having a role in transcriptional regulation. The observations may be of use in altering the expression of the gene in other species including humans.

MATERIALS AND METHODS

Blood samples were collected from jugular vein of buffalo and Vechur cattle (ten each), maintained in the University Buffalo farm and VechurCattle Conservation Trust, College of Veterinary and Animal Sciences,Mannuthy,respectively. Genomic DNA was isolated using phenol chloroform extraction method (SambrookandRussell, 2001). DNA were amplified at the promoter region of ATP citrate lyase gene using primers designed from the whole genome sequences of *Bostaurus*available in the data bank.

Amplification of the promoter region

The primer sequences were5'AACAGGACTTGCCAACCAGA 3' (F) and 5' TGCCACCTCAGTCTTTCCTCT 3'(R). Each reaction was carried out in a total volume of 25 µl containing 0.2 mMdNTPs, 1.5 mM$MgCl_{2,}$ 20 pM ofeach primer, 1.2 UTaqDNA polymerase, 1 µl template DNA (~100ng) with an initial denaturation for 5 minutes at 95ºC, followed by the cycle parameters, 95ºC for 1 minute (denaturation), 60.7ºC for 1 minute. (annealing), and 72ºC for 2 minutes. (extension) for 35 cycles with a final extension for 5 minutes at 72ºC.

Purification of PCR products, sequencing and analysis

The PCR products were electrophoresed in low melting point (1.5%)agarose along with 100 bp ladder as molecular size marker. The fragments were extracted from agarose gel using GeneJET™ Gel Extraction Kit (Fermentas Life Sciences, Lithuania). Each smaple was sequenced three times before submitting in NCBI data bank. Sequencing was carried out at SciGenomPvt. Ltd, Kakkanadu, Ernakulam.The sequences obtained wereanalysed and compared with data available in Gen Bank using the programme clustalW.

RESULTS AND DISCUSSION

PCR amplification and sequence analysis

Amplification of the promoter region of ACLY gene generated a products of size between 700 and 800 bp (Figure 1). The products were purified, sequenced and analysed. Multiple sequence alignment of the sequences is shown in Figure 2.

A consensus TATA box, which is considered as the core promoter for transcription initiation, to which RNA polymerase II binds, is found to be absent in the obtained sequences whereas,TATA like sequence such as TATAT and TATATA is seen in between 18 to 115 region.This agrees with the finding ofKim *et al.* (1994) and Park *et al.* (1997) in rat and human respectively. The AT rich region can facilitate easy unwinding of DNA due to weak interaction between the bases than GC during

initiation of transcription.

A CAAT sequence is located at 618 position in buffalo. Two such sequences are present in the promoter region of Vechur cattle at positions 627 and 497. It has been reported that CAAT box is present at 87 in rat (Kim *et al*., 1994) and at 92 (Park *et al*., 1997) in human, at a position near to the transcription initiation site. The CAAT box is normally situatedat about 70 bp upstream of the transcription initiation site, whichsignals the binding of RNA polymerase. In the present study, the location of CAAT box at a site, far away from the transcription initiation, might be a contributing factor for the low expression of this enzyme in ruminants.

Cyclic AMP responsive element (CRE) like sequence is present at 50 to 42 region, at a site near to transcription initiation. A single base mutation is observed from the consensus sequence in both the species. One such sequence is present at position 1283 in rat and at 1428 in human. Several researchers have reported that binding of cyclic AMP regulatory protein decreases the expression of lipogenic enzymes in liver (Kim *etal*., 1994; Paulauskis and Sul, 1989). In Buffalo and Vechur cattle, presence of this element, near to the transcription initiation site, might play a role in the suppression of the gene in ruminants.

Fat Specific Element (FSE) has a role in the expression of ACLY in adipocytes. A sequence

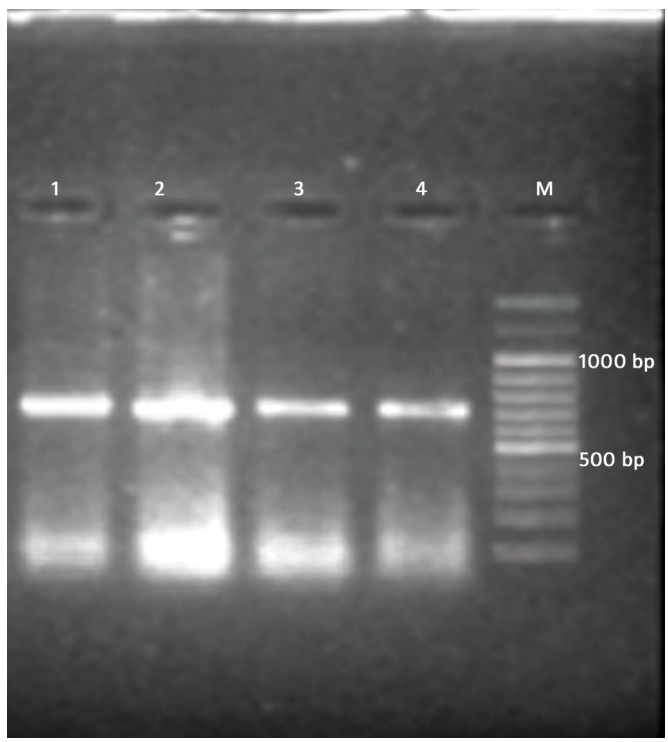

Figure 1. Amplified product of ACLY promoter in buffalo and vechur cattle.
Lane - 1 and 2(Amplified product in buffalo), 3 and 4(Amplified product in vechur cattle), both having a size between 700 and 800 bp.

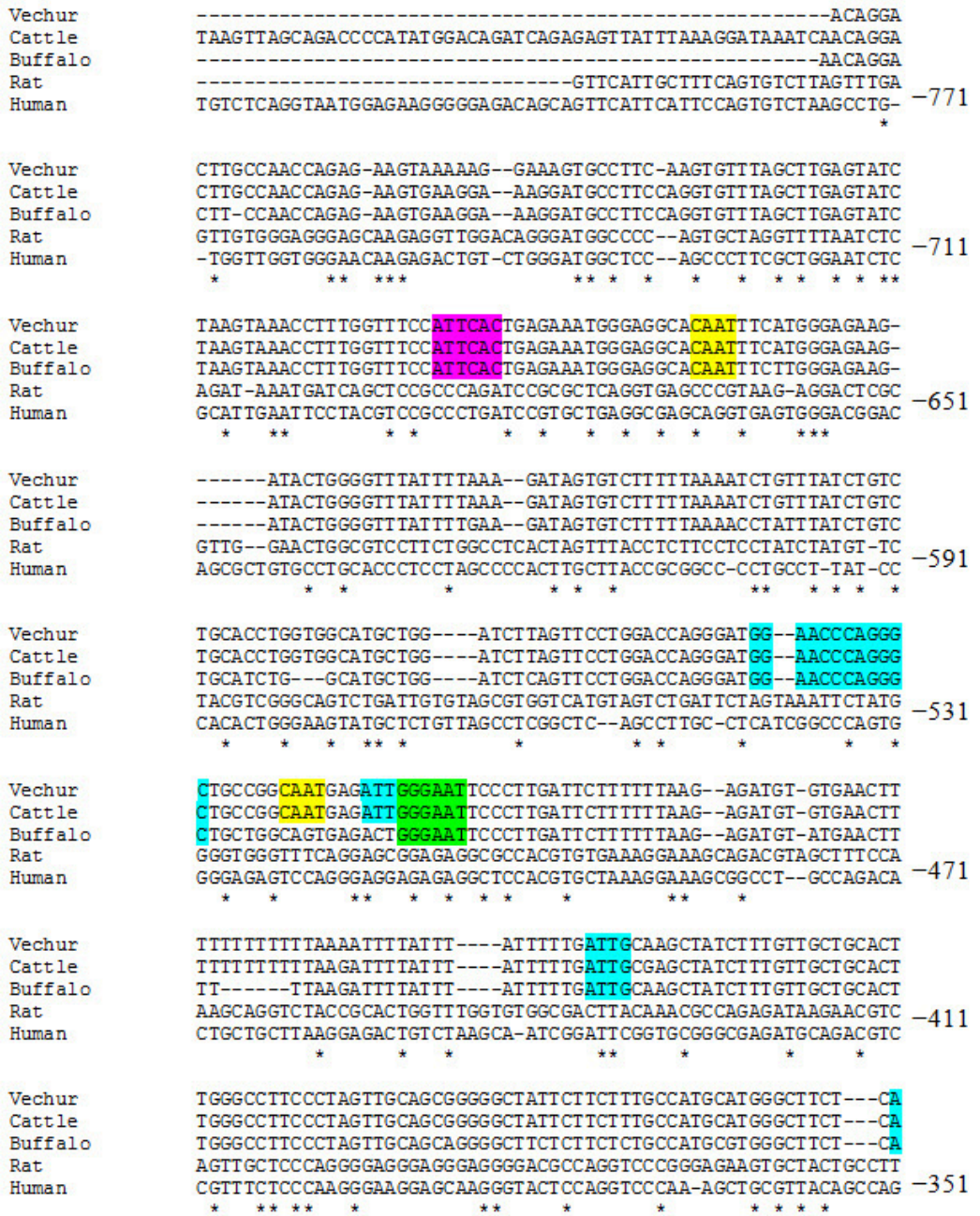

Figure 2. Multiple sequence alignment of ACLY promoter region of vechur, cattle, buffalo, rat and human using the programme ClustalW.

Green - Initiation site, TATA like sequences, NFKB and Enhancer core
Yellow - CAAT box, Special protein binding sites.
Blue - GRE and Inverted CAAT box
Pink - CRE, E- box and HNF
Grey - FSE
Green and Blue - NFKB

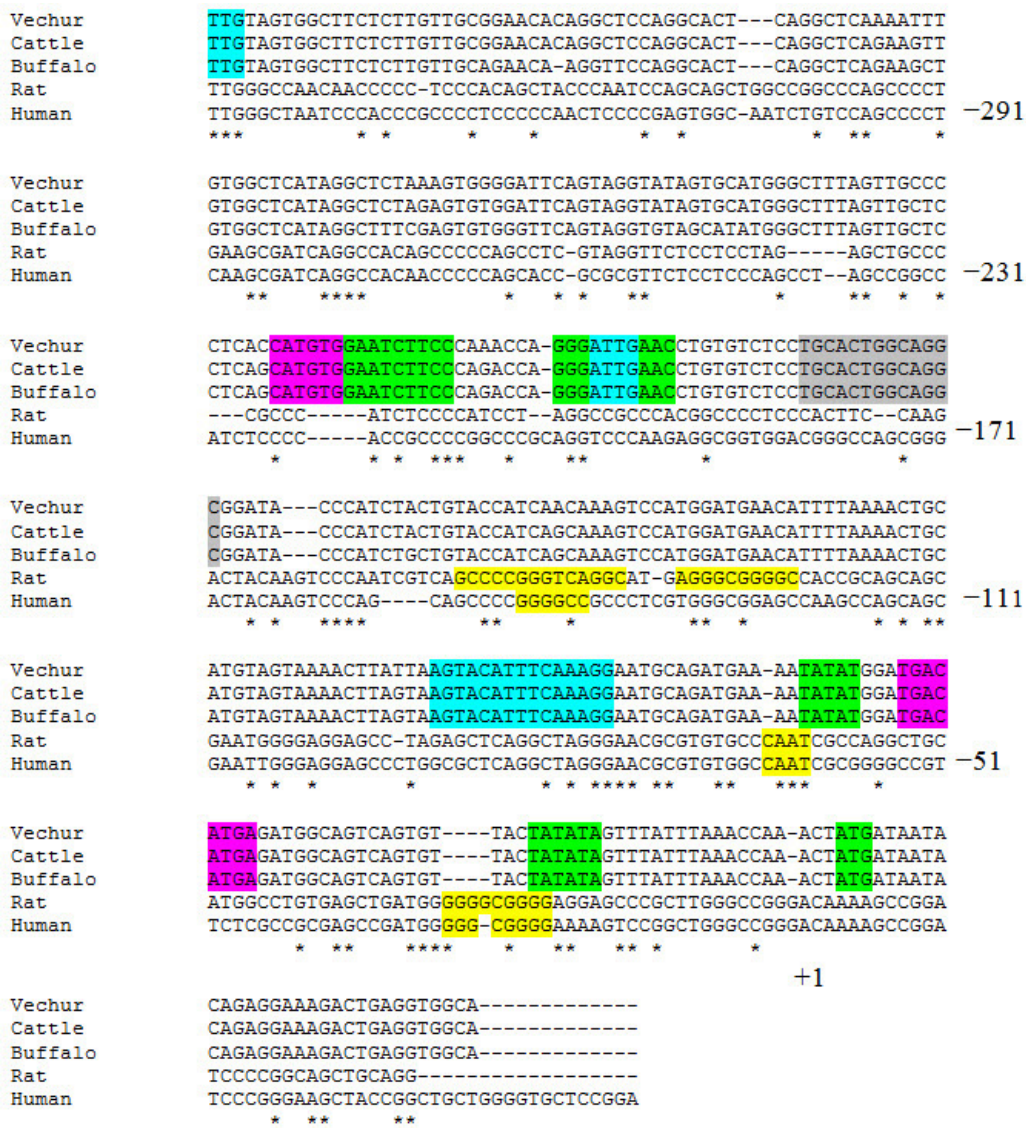

Figure 2. Multiple sequence alignment of ACLY promoter region of vechur, cattle, buffalo, rat and human using the programme ClustalW. (Cont.)

Green - Initiation site, TATA like sequences, NFKB and Enhancer core

Yellow - CAAT box, Special protein binding sites.

Blue - GRE and Inverted CAAT box

Pink - CRE, E- box and HNF

Grey - FSE

Green and Blue - NFKB

similar to that is present in the promoter of both buffalo and Vechur cattle at position 174 to 161 but two such elements are seen in rat at 370 and 175and in human at 842 and 1869 (Kim *et al*., 1994 and Park *et al*., 1997). Low expression of ACLY in ruminants could also be correlated to this observation.

Site similar to Hepatocyte Nuclear Factor I binding site (HNF) is seen at position 644 to 638 in buffalo and 653 to 647 in Vechur cattle with single base pair mutation from the consensus sequence. In rat and human it is present at positions 1654 and 1837 respectively. HNF is involved in tissue specific expression of the gene (Courtois*et al*., 1987 and Kim *et al*., 1994) and variation in the position of this site might be responsible for the differential expression of this gene in ruminants and non-ruminants.

Presence of inverted CAAT box (ATTG) is another important observation, which is located at three sites in buffalo and four sites in Vechur cattle. Inverted CAAT Box is an essential element for mediation of transcriptional regulation throughcAMP (Rangan*et al*., 1996 and Roder*et al*., 1999). Kim *et al*. (1994) reported that Cyclic AMP decreases the expression of lipogenic enzymes in liver.

Other important sequences observed are Enhancer core (484 to 479 in Buffalo and 491 to 485 in Vechur cattle), Nuclear factor-kB (211 to 201 and 195 to 182 in both the species)and an E box (216 to 210 in both the species). Enhancer core, the core promoter not only mediates the initiation of transcription, but also functions as a regulatory element (Kudo*et al*., 2000). Nuclear factor-kBconsists of a family of transcription factors that play critical roles in inflammation, immunity, cell proliferation, differentiation, and survival (Oeckinghaus and Ghosh, 2009). Upstream stimulatory factor USF-1 and USF-2, are

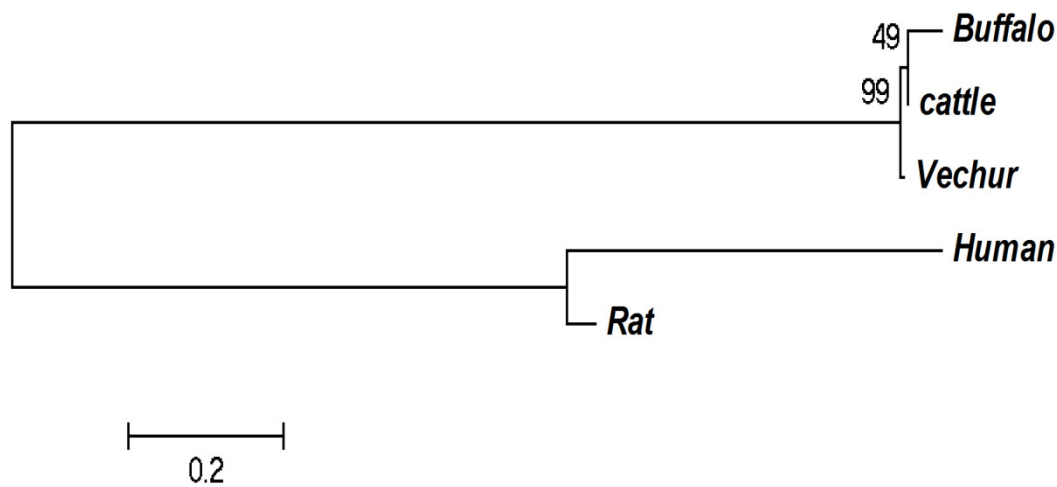

Figure 3. Phylogenetic tree of ACLY promoter of Vechur cattle, Cattle, Buffalo, Rat and Human. (Neighbor joining analysis usingMega4, where branch length represents the degree of genetic divergence, and the scale bar corresponding to the number of base changes per site).

major components of protein complexes that bind to E-box motif and it act as an insulin response sequence in differentiating adipocytes (Travers *et al*., 2001).

The special protein binding sites (Sp sites) and GC rich regions which enhances the expression of gene (Rolland *et al*., 1996; Roder*et al*., 1999 and Liu *et al*., 2000) is absent in the promoter of ACLY in Buffalo and Vechur cattle whereas, several such sequences are present in a region between 310 to 30 in rat and human (Kim *et al*., 1994 and Park *et al*., 1997). Absence of these sites might be another reason for the low expression of this gene in these species.

Phylogenetic analysis of the nucleotide sequences using Bootstrap kimuraneighbor joining tree (MEGA version4, Arizona State University, USA) revealed clustering of buffalo, Vechur cattle and Cattle on one clade and Rat and Human delineated from other species and clustered out on a separate clade (Figure 3).

Thus, analysis of the promoter region of ACLY gene in buffalo and Vechur cattle revealed the presence of various consensus sequences for the binding of specific proteins regulating its expression.

Absence of TATA box, SP sites and GC rich region and the presence of specific sequence motifs such as CRE, CAAT box and inverted CAAT box, its position and number play a very crucial role in the expression of the gene and probably contribute to the low level expression of ACLY in ruminants.

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