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Research article

Molecular characterisation of coding regions of *HIF-1a* gene in Vechur cattle by cDNA sequencing

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ABSTRACT

Hypoxia-inducible factor (HIF)-1α is a transcription factor stabilized by hypoxia by inducing or suppressing the homeostatic regulatory gene expression, enabling tissues and cells to survive despite fluctuations in environmental circumstances. As the name implies, hypoxia-inducible factor-1 is secreted not only as a cellular response to hypoxia but also in heat stress and oxidative stress. The goal of this work was to determine the molecular characterisation of the *HIF-1α* gene coding region as well as the differences in HIF-1αprotein primary structure between Vechur cattle and other cattle breeds in the online databases. Total RNA was isolated from blood samples of 6 Vechur cattle using the trizol reagent method, and full-length c sequences of the *HIF-1α* gene were sequenced. The base pair length of composite HIF-1αcDNA of Vechur cattle and encoding ORFis 3956 bp and 2469 bp respectively. The 5′UTR was recognized to be 279 bp in length. The start codon was identified at nucleotide 280–282, the stop codon UGA at 2746–2748 bp and a 1208 bp 3′UTR which included a poly-A tail of 27 adenine residues. In a comparative analysis of the cDNA, point transitions causing guanine to adenine (G>A) changes at 1211th and 2699th positions were noticed as a heterozygous condition in the whole 3956 bp sequence. These two SNVs in the coding regions were responsible for two amino acid changes in the deduced 823 amino acid sequence. Since the predicted amino acid arginine had been replaced with lysine at 311th and 807th positions, it showed 99.76 percent sequence identity with *Bos taurus*. The phylogenetic tree revealed that the HIF-1α protein of Vechur cattle had a lesser evolutionary distance from the same gene of related species emphasising the highly conserved nature of this particular protein. This structural variation observed in the present study should be evaluated on a larger population to assess its functional relevance for thermo-tolerance.

1. Introduction

Vechur cattle (*Bosindicus*), one of the smallest cattle breeds in the world, is the only native cattle breed of Kerala, the southernmost state in India. These native animals have acquired geo-climatic resilience through natural selection and have gained acceptance among the farming community. Hypoxia-inducible factor-1α (*HIF-1α*) gene is one of the most important regulatory genes of homeostasis. Stressors like heat, hypoxia, reactive oxygen species, and nitric oxide limit HIF-1*α*degradation and promote transcription thereby allowing HIF-1*α* to accumulate. HIF-1*α*, in turn, binds to HIF-1 to form the activated HIF-1 heterodimer, thereby triggering multiple cellular pathways associated with stress responses and acclimatization (Treinin et al., 2003; Ely et al., 2014). This enables tissues and cells to survive despite fluctuations in environmental circumstances.

HIF-1 is a dimeric protein (HIF-1α and HIF-1β) and each subunit of HIF-1 is a part of the basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) family and includes two PAS domains, PAS-A and PAS-B (Ziello et al., 2007). The amino terminus of HIF-1α constituted by 1–390 amino acids contains bHLH and PAS domains which are vital for DNA binding and dimerization (Jiang et al., 2011). The carboxy terminus possesses an oxygen-dependent degradation domain (ODDD) which is responsible for the oxygen-dependent instability of HIF-1α (Soni and Padwad, 2017). The N-terminal transactivation domain (NTAD) overlaps with the ODD domain and for complete HIF activity, the C-terminal transactivation domain (CTAD) interacts with co-activator like p300/CBP, regardless of

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protein stabilization (Masoud and Li, 2015). The hypoxia response element (HRE; 5′-RCGTG-3′) of promoter regions of regulatory genes was first identified in the 3′ enhancer region of the gene for erythropoietin (EPO), a hypoxia-induced hormone helping the proliferation of RBCs (Semenza et al., 1991; Chao et al., 2021).

Under normoxic conditions, the prolyl hydroxylases hydroxylate the conserved proline residues (pro 402 and pro 564) and acireductone dioxygenase-1 (ARD-1) acetylate the Lysine 532 within the ODDD of HIF-1α subunit (Bruick and McKnight, 2001; Epstein et al., 2001). This post-translational modification makes it a recognition element for the von Hippel-Lindau tumour suppressor protein (pVHL), a part of the protein-ubiquitin ligase complex, thus tagging the subunit for sudden degradation (Ivan et al., 2001; Jaakkola et al., 2001; Yu et al., 2001). During hypoxia, HIF-1α translocates from the cytoplasm to the nucleus and dimerises with ARNT (aryl hydrocarbon receptor nuclear translocator or β subunit), and this heterodimer becomes a transcription factor causing the promotion of target gene transcription. Co-activators such as CREB-binding protein/p300 (CBP/p300) on interaction with CTAD, cause recruitment of transcriptional machinery, thus leading to the full induction of the actions of HIF-1 (Carrero et al., 2000; Kung et al., 2000; Gu et al., 2001, Ziello et al., 2007; Masoud and Li, 2015; Chao et al., 2021).

The study of breeds, using molecular techniques is very important and useful for their characterisaton (Mohammadi et al., 2009; Mohammadabadi, 2021). Conservation of genetic diversity in animal species requires the proper performance of conservation superiorities and sustainable handling plans that should be based on universal information on population structures, including genetic diversity resources among and between breeds (Mohammadabadi et al., 2010; Mohammadabadi et al., 2017). Genetic diversity is an essential element for genetic improvement, preserving populations, evolution and adapting to variable environmental situations (Zamani et al., 2015; Mohammadabadi et al., 2021). On the other hand, the determination of gene polymorphism is important in farm animals' breeding (Koopaei et al., 2012; Gooki et al., 2019) in order to define genotypes of animals and their associations with productive, reproductive and economic traits (Nassiry et al., 2005; Norouzy et al., 2005; Gholamhoseini Gooki et al., 2018). The focus of this research was to characterise and compare the sequences of the coding regions of the *HIF-1 α*gene of Vechur cattle with the available sequences of the same gene in the database and also to identify the predicted amino acid sequence of this particular protein. So this study was designed to understand the genetic and molecular reasons for their homeostatic resilience in the hot humid tropical climate of the state.

2. Materials and methods

2.1. Sample collection

Blood samples were collected from six Vechur cattle maintained at University Livestock Farm, Mannuthy, Kerala, India. Two milliliters of blood were collected from the jugular vein of each animal into EDTA-coated vacutainer tubes. The experiment was conducted according to the established animal welfare guidelines of the ethical committee of the Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala, India and it was confirmed that the study complies with all regulations of the ethical committee.

The blood samples were kept on ice and brought to the laboratory and processed within 10 min.

2.2. Isolation of total RNA

After ice-cold RBC lysis buffer treatment and phosphate-buffered saline washing, total RNA was isolated using Trizol reagent (Invitrogen, USA). The integrity of the extracted RNA was assessed by electrophoresis on one percent agarose gel in a 1X TAE buffer (Figure 1). Quality analysis and quantification were done by measuring the absorbance of the samples at 260 and 280 nm using a NanoDrop spectrophotometer (Thermo Scientific, USA). Samples with an A260/A280 ratio between 1.7 and 2.0 were used for further processing (Sambrook et al., 2001 and Manchester, 1996).

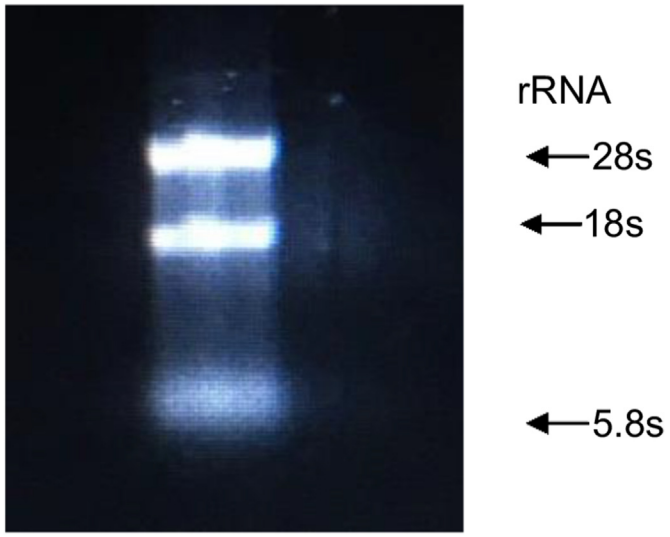

Figure 1. Extracted RNA on agarose gel.

Complementary DNA was synthesized using the Verso cDNA synthesis kit (Thermo Scientific-AB1453/B, USA) as per the manufacturer's instructions. The concentration, purity, and quality of cDNA were checked in a NanoDrop spectrophotometer (NanoDrop TM, 2000C).

2.3. Sequence amplification

*HIF-1a*gene of *Bos taurus*as a reference to design the primers was collected from NCBI Gen Bank (Gen Bank NM_174339.3). Using Primer3 and primer blast software ten sets of overlapping primers (Table 1) were designed for the 3956 bp long, *HIF-1a* sequence which has 17 exons, located on chromosome 10 (Region: 73836385–73881302). PCR Primer Stats in Sequence Manipulation Suite was used to check the suitability of each set of PCR primers.

Gradient polymerase chain reaction (PCR) was performed for all the ten sets of primers at annealing temperatures ranging from 59.4 °C to

Table 1. Sequences of primers used in PCR.

Sl. No.	Primer	Primer sequence	Product size (bp)
1	Forward	CTGAGGGGACGCGAGGAT	424
	Reverse	TATGGGGGAGTGGCAACTGA	
2	Forward	CAGCCAGATCTCGTCGAAGT	468
	Reverse	TTCTCCCCCGGCTAGTTAGG	
3	Forward	GCCGGGGGAGAACTATGAAC	292
	Reverse	GAGCGGCCCAAAAGTTCTTC	
4	Forward	AGAAGAACTTTTGGGCCGCT	405
	Reverse	GGGGCCAGCAAAGTTAAAGC	
5	Forward	GCTTTAACTTTGCTGGCCCC	631
	Reverse	CTTGCATTTGAGTGGGCTGG	
6	Forward	CCAGCCCACTCAAATGCAAG	585
	Reverse	CTGGTCAGCTGTGGTAGTCC	
7	Forward	TTCCATCTCCTCCCCACGTA	672
	Reverse	TTGTGCAGTATTTGTAGCCAGG	
8	Forward	GGACACTTGGCTCATTACC	818
	Reverse	AACAGGGTGGGCAGAACATT	
9	Forward	TTCTGCCCACCCTGTTGGTA	290
	Reverse	GAAGATCCAACCACAAAGAGCA	
10	Forward	ACATCTTGTTTTTCTATGTGCATTGT	119
	Reverse	TGGTCCACAGAAGATGTTTATTTGA	

Table 2. PCR reaction conditions for each primer.

Cycle step	Time (s)	The temperature of each 10 sets of primers									
		1	2	3	4	5	6	7	8	9	10
Initial denaturation	180	95	95	95	95	95	95	95	95	95	95
Denaturation	30	95	95	95	95	95	95	95	95	95	95
Annealing	30	64.6	64.1	63	61	65	59.4	60.1	60.1	61.7	64.8
Extension	60	72	72	72	72	72	72	72	72	72	72
From denaturation, repeated 35 cycles											
Final extension	300	72	72	72	72	72	72	72	72	72	72
Hold	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞

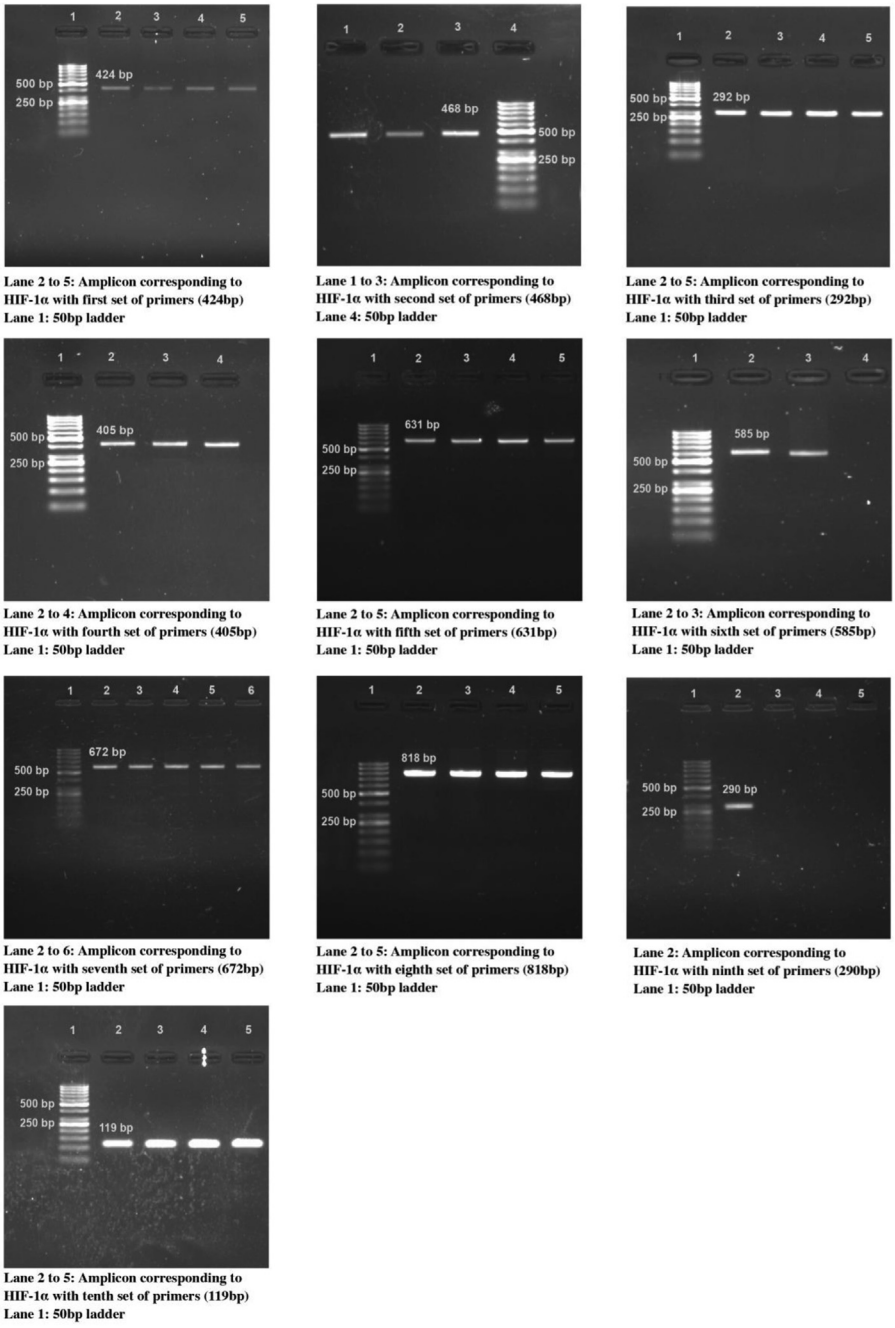

Figure 2. Gel images of the ten amplicons of *the HIF-1α* gene.

Table 3. BLASTn results and comparisons.

Sequence Id	Scientific name	Nucleotide variations (">" represents "to")	Position of change	Percent identities
NC-037337.1	*Bos taurus* mRNA	G>A G>A	1211 bp 2699 bp	99.5
AB018398.1	*Bos taurus* complete coding sequence	G>A G>A A>G	1211 bp 2699 bp 2095 bp	99.88
XM-019969024.1	*Bos indicus*	G>A G>A Deletion of C	1211 bp 2699 bp 190 bp	99.76
XM-027554265.1	*Bos indicus* × *Bos taurus* predicted transcript variant	G>A G>A	1211 bp 2699 bp	99.44
AY621118.1	*Bos grunniens* complete coding sequences	T>C G>A T>C C>T G>A G>C Insertion of G G>C Insertion of G Insertion of C Insertion of T G>A Insertion of A A>T Insertion of A Insertion of A	702 bp 1211 bp 1863 bp 1923 bp 2699 bp 2727 bp 2969 bp 2968 bp 3046 bp 3053rd bp 3090thbp 3111th bp 3125th bp 3497th bp 3512nd bp 3528 bp	99.55
XM-005890693.1	*Bos mutas* transcript variant	C>T T>A T>C G>A T>C C>T G>A A>G T>C	105 bp 111 bp 702 bp 1211 bp 1863 bp 1923 bp 2699 bp 2868 bp 3591 bp	99.76
XM-025295403.1	*Bubalus bubalis*	A>G T>C G>C T>C T>C T>C A>G G>A G>A C>A C>T G>A C>T G>A C>T G>A C>T T>C A>T Deletion of A	13 bp 71 bp 124 bp 192 bp 465 bp 701 bp 933 bp 1188 bp 1211 bp 1293 bp 1575 bp 1626 bp 2103 bp 2699 bp 2936 bp 2962 bp 2966 bp 3168 bp 3547 bp 3632 bp	99.53

65 °C in two sets of reactions (cycling conditions given in Table 2). Amplified PCR products were subjected to electrophoresis in two percent agarose gel in a submarine electrophoresis apparatus (Hoefer, USA). The temperature at which single specific bands were obtained in gel electrophoresis was fixed as the annealing temperature for each pair of primers (Figure 2).

2.4. Sequence analysis

2.4.1. Nucleotide sequence analysis of HIF-1a in Vechur cattle

The amplicons obtained consequent to PCR with the primers targeting full coding regions of *the HIF1a* gene of Vechur cattle were purified and sequenced at Agri Genome Labs Private Limited, Cochin, Kerala, India using Sanger's dideoxy nucleotide chain termination method. Bidirectional sequencing was done with both forward and reverse primers. The reverse complement of the reverse sequence was obtained from the Sequence Manipulation Suite Reverse Compliment program (https://www.bioinformatics.org/sms2/rev_comp.html). The forward sequence and the reverse complement of the reverse sequence were aligned using EMBOSS MERGER (http://www.bioinformatics.nl/cgi-bin/emboss/merger) and fragments of genes were aligned using Align Sequences Nucleotide BLAST program (www.blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&LINK_LOC=align2seq).

```
Query: unnamed protein product Query ID: lcl|Query_454907 Length: 823


>hypoxia-inducible factor 1-alpha [Bos taurus]
Sequence ID: NP_776764.2 Length: 823
>Hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)
[Bos taurus]
Sequence ID: AAI26623.1 Length: 823
>TPA: hypoxia-inducible factor 1-alpha [Bos taurus]
Sequence ID: DAA25052.1 Length: 823
Range 1: 1 to 823

Score:1711 bits(4431), Expect:0.0,
Method:Compositional matrix adjust.,
Identities:821/823(99%), Positives:823/823(100%), Gaps:0/823(0%)

Query  1    MEGAGGANDKKKISSERRKEKSRDAARSRRSKESEVFYELAHQLPLPHNVSSHLDKASVM  60
Sbjct  1    ............................................................  60

Query  61   RLTISYLRVRKLLDAGDLDIEDEMKAQMNCFYLKALDGFVMVLTDDGDMIYISDNVNKYM  120
Sbjct  61   ............................................................  120

Query  121  GLTQFELTGHSVFDFTHPCDHEEMREMLTHRNGLVKKGKEQNTQRSFFLRMKCTLTSRGR  180
Sbjct  121  ............................................................  180

Query  181  TMNIKSATWKVLHCTGHIHVYDTNSNQSQCGYKKPPMTCLVLICEPIPHPSNIEIPLDSK  240
Sbjct  181  ............................................................  240

Query  241  TFLSRHSLDMKFSYCDERITELMGYEPEELLGRSIYEYYHALDSDHLTKTHHDMFTKGQV  300
Sbjct  241  ............................................................  300

Query  301  TTGQYRMLAKKGGYVWIETQATVIYNTKNSQPQCIVCVNYVVSGIIQHDLIFSLQQTECV  360
Sbjct  301  ..........R.................................................  360

Query  361  LKPVESSDMKMTQLFTKVESEDTSSLFDKLKKEPDALTLLAPAAGDTIISLDFGSNDTET  420
Sbjct  361  ............................................................  420

Query  421  DDQQLEEVPLYNDVMLPSSNEKLQNINLAMSPLPASETPKPLRSSADPALNQEVALKLEP  480
Sbjct  421  ............................................................  480

Query  481  NPESLELSFTMPQIQDQPASPSDGSTRQSSPEPNSPSEYCFDVDSDMVNEFKLELVEKLF  540
Sbjct  481  ............................................................  540

Query  541  AEDTEAKNPFSTQDTDLDLEMLAPYIPMDDDFQLRSFDQLSPLENSSTSPQSASTNTVFQ  600
Sbjct  541  ............................................................  600

Query  601  PTQMQEPPIATVTTTATSDELKTVTKDGMEDIKILIAFPSPPHVPKEPPCATTSPYSDTG  660
Sbjct  601  ............................................................  660

Query  661  SRTASPNRAGKGVIEQTEKSHPRSPNVLSVALSQRTTAPEEELNPKILALQNAQRKRKIE  720
Sbjct  661  ............................................................  720

Query  721  HDGSLFQAVGIGTLLQQPDDRATTTSLSWKRVKGCKSSEQNGMEQKTIILIPSDLACRLL  780
Sbjct  721  ............................................................  780

Query  781  GQSMDESGLPQLTSYDCEVNAPIQGSKNLLQGEELLRALDQVN  823
Sbjct  781  ..........................R.................  823
```

Figure 3. Comparison of HIF-1α protein sequences between Vechur cattle and *Bos taurus* by BLASTp.

2.4.2. Comparison of HIF-1a between Vechur cattle and other published sequences of different breeds of cattle

The *HIF-1a* sequences of Vechur cattle were compared with other published sequences of the coding regions of the same gene available in online databases, using the bioinformatics tool BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch), to analyse their similarity. Using the Expasy bioinformatics tool (https://web.expasy.org/translate/), the DNA sequence was translated to its corresponding amino acids sequence and the translated protein basted was compared using the Protein Blast tool (https://blast.ncbi.nlm.nih.gov⟩ Blast) with other published amino acid sequences available in online databases for analysing homology. The bioinformatics tool Uniprot (https://www.uniprot.org) was used for the identification of the HIF-1α protein domain.

2.5. Phylogenetic analysis

The amino acid sequences of the HIF-1α protein of related species in FASTA format were obtained from NCBI (https://www.ncbi.nlm.nih.gov/). All amino acid sequences collected were aligned by Multiple

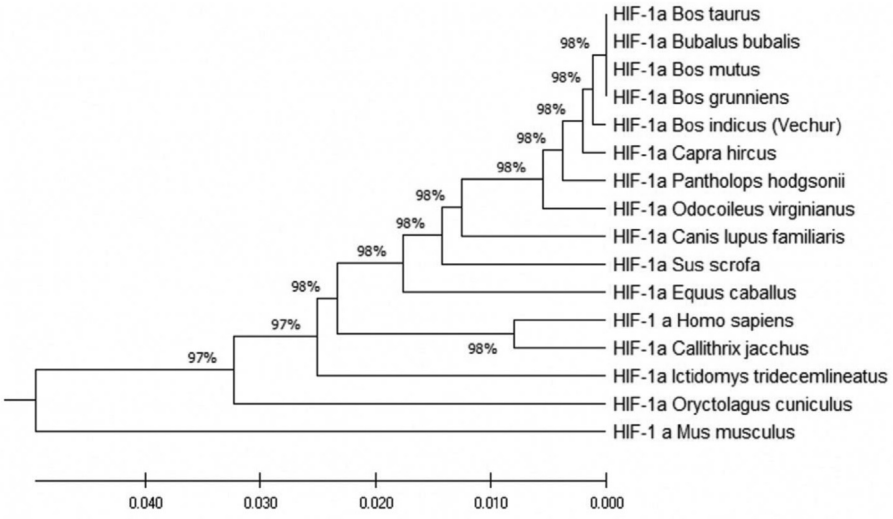

Figure 4. Phylogenetic tree.

Alignments using Fast Fourier Transform (MAFFT) (https://www.ebi.ac.uk/Tools/msa/mafft/) bioinformatics tool. The phylogenetic tree and Sequence distance chart were constructed by Molecular Evolutionary Genetics Analysis (MEGA) (https://www.megasoftware.net/). The neighbour-joining method was performed using deduced amino acids for the phylogenetic tree and the Poisson correction method was used for assessing the evolutionary distance.

2.6. Protein modeling

Using the Swiss model (https://swissmodel.expasy.org/) protein was recreated using 4ZPRB as a reference structure obtained from the protein data bank (PDB). Ramachandran plot server, zlab online tool (https://zlab.umassmed.edu/bu/rama/index.pl) was used to analyze protein structure with Ramachandran plot.

3. Result

The full-length HIF-1α cDNA sequence of Vechur cattle has been submitted to GenBank and accession No. MN783015 was obtained.

3.1. Nucleotide analysis

HIF1α sequence was analysed for the open reading frame (ORF) using Expasy, and a 2469 bp ORF (280–2748 bp) sequence was identified. The 5′UTR was recognised to be 279 bp in length. The start codon was identified at nucleotide 280–282, and the stop codon UGA at 2746–2748 bp. A 1208 bp 3′UTR which included a poly-A tail of 27 adenine residues was also identified.

BLASTn displayed 99.95 percent homology with *Bos taurus* sequence (NC_037337.1) with a 3954 bp identity out of 3956 bp subjected to analysis. G > A changes were observed at the 1211th and 2699th positions of the 3956 bp sequence. The *HIF-1α* open reading frame has 2469 bp that has homology with *HIF-1α* of other species. BLASTn result is detailed in Table 3 The sequence identities of Vechur*HIF-1α* and that of other species are *Mus musculus* 87.24 percent (U59496.1) and *Homo sapiens* 93.72 percent (U22431.1). The sequence of *HIF-1α* revealed variations at positions 1211 and 2699.

3.2. Protein sequence analysis and its comparison with published sequences of different breeds of cattle

Using Expasy bioinformatic tool, cDNA sequences were translated to their corresponding amino acid sequences which are shown in Figure 3. The open reading frame of HIF-1 *α* is 2469 bp and codes for 823 amino acid residues (Hara et al., 1999).

Protein Blast displayed 99.76 percent homology with *Bos taurus* (NP_776764.2) and 821 amino acids were identical out of the 823 amino acids subjected for analysis. Two changes, both arginines to lysine, * were noticed at positions 311 and 807. The same changes were observed in *Bos indicus* (XP_019824583.1) and *Bos indicus*×*Bos taurus* isoform X2 (XP_027410067.1) at the same positions. The sequence identities of Vechur HIF-1*α* and that of other species are *Bos indicus* 99.76 percent (XP_019824583.1), *Bos indicus*× *Bos taurus* isoform X2 99.76 percent (XP_027410067.1), *Bos mutus* 99.63 percent (ELR60049.1), *Bos grunniens* 99.27 percent (Q0PGG7.1), *Bubalus bubalis* 96.70 percent (XP_025151188.1) and *Homo sapiens* 94.92 percent (AAC685668.1).

3.3. Phylogenetic analysis

HIF1α sequence of sixteen species of animals was downloaded and used to construct a phylogenetic tree (Figure 4). Except for Vechur cattle, all *Bovidae* family members were found to be included in the same group. The unique sequence variation of the Vechur*HIF-1α* gene from other members of the *Bovidae* family has resulted in this divergence from that of other species. All except *Mus musculus* branched under the same node. The phylogenetic tree revealed that the evolutionary distance between

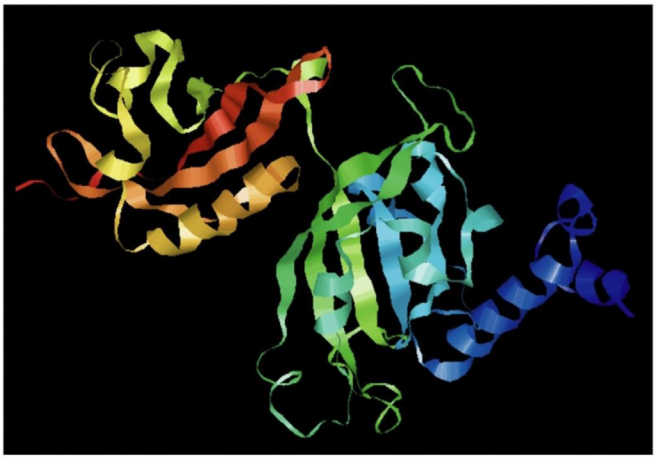

Figure 5. 3D structure of HIF-1α protein

the related species is very less for this particular protein and that emphasised the highly conserved nature of HIF-1*α* (Jiang et al., 2011). The sequence distance chart displayed maximum divergence with *Mus musculus* (10.12 percent) and least divergence with *Bos taurus*, *Bos grunniens*, *Bos mutus*, and *Bubalus bubalis* (0.24 percent).

3.4. Protein modelling

The protein structure was modelled using *Mus musculus* HIF-1α protein structure as a reference (accession number-4ZPRB) from the protein data bank of the online modeling tool SWISS-MODEL (Figure 5). This reference structure showed only 41 percent query coverage because of the reason that the crystal structure was prepared for a segment containing 345 amino acids. But this structure showed 96.81 percent sequence identity with the amino acid sequence deduced from this study and 4ZPRB was the best structure selected by the SWISS-MODEL tool.

Ramachandran plot server, zlab online tool was used to analyze protein structure with Ramachandran plot (Figure 6) and the result showed as follows:

Number of residues in the favoured region: 1234 (72.206%).
Number of residues in the allowed region: 294 (17.203%).
Number of residues in outlier region: 181 (10.591%) The percentage of residues in various locations, such as the favoured region, allowed region, and outlier region, are depicted in the plot created by the rampage online tool.

Highly preferred observations are shown as green crosses: 1234 (72.206%).
Preferred observations are shown as brown triangles: 294 (17.203%).
Questionable confirmations are shown as red circles: 181 (10.591%).

4. Discussion

The disclosed Vechur cattle *HIFα* gene sequence is nearly identical (99.88 percent) to previously published *HIF-1α* mRNA nucleotides of *Bos taurus*. The two identified single nucleotide variations (SNVs) (G1211A and G2699A) in the coding sequences of *HIF-1α* in Vechur cattle are the first reported SNVs of *HIF-1α* in the bovine species. Such

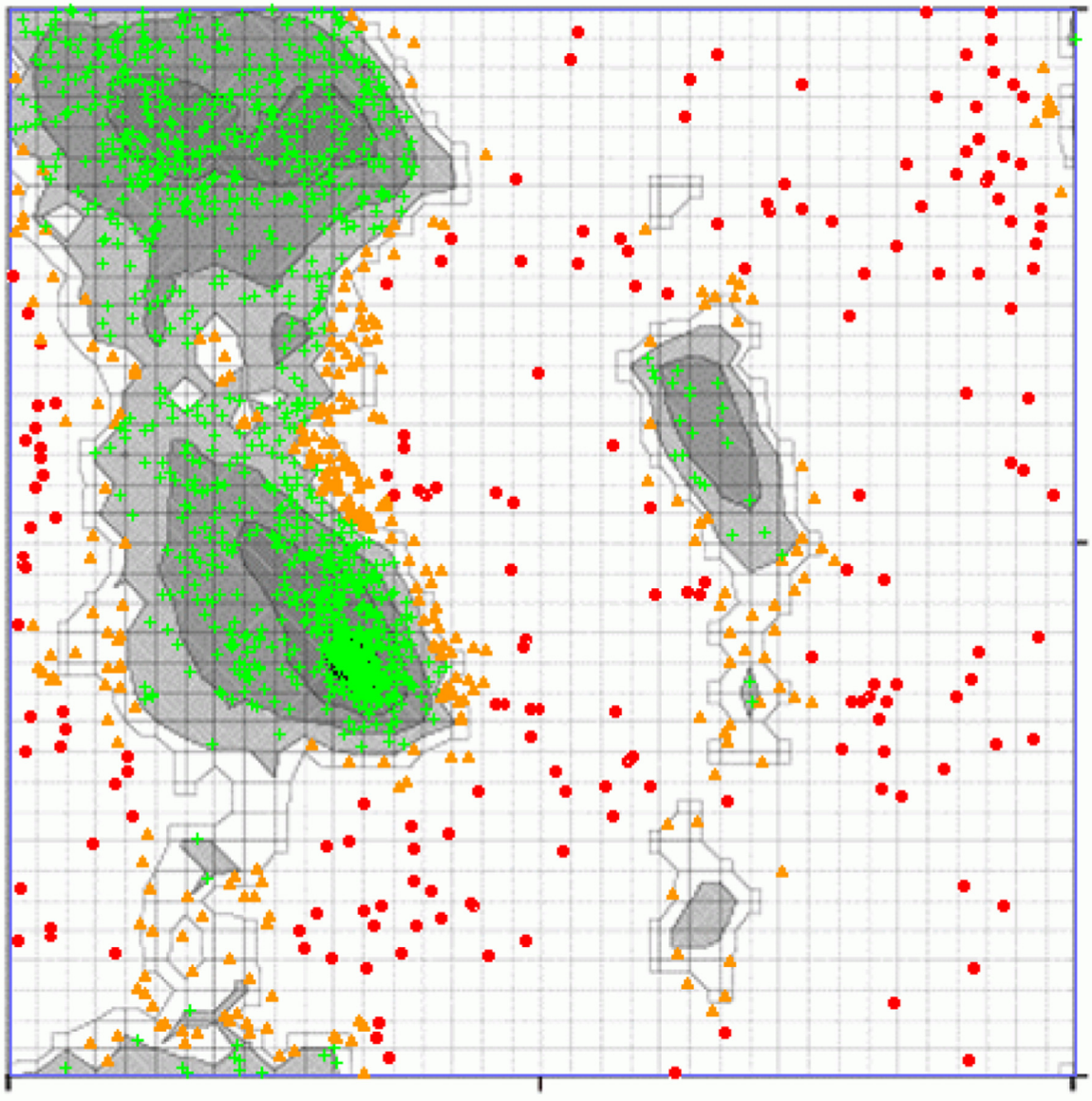

Figure 6. Ramachandran plot: Color-coded chart decodes: - Black, Dark grey, and Light grey represent highly preferred conformations. White with a black grid represents preferred confirmations. White with a grey grid represents questionable confirmations.

point variations (transitions and transversion) in the *HIF-1a* gene are not detected in any other domestic animal breeds to date, but SNPs in other regions (C1772T, G1790A, and C111A) were detected in human breast cancer patients (Apaydin et al., 2008). These SNVs resulted in two amino acid changes-both arginine to lysine. One of the two lysine molecules was found at the 311th position after the PAS-B domain and another one in the middle of the CTAD at the 807th position, in place of arginine. The predicted amino acid sequences of Vechur cattle HIF-1α protein showed strong similarities with other *Bos taurus* primary sequences except for the two amino acid variations. The BLAST result indicated the highly conserved nature of the HIF-1α protein and also emphasised the importance of this protein in homeostasis. In the phylogenetic analysis, the HIF-1α protein sequence of Vechur cattle was clustered separately because of the difference in the amino acid sequence in comparison to the amino acid sequences of similar species of animals.

HIF-1α protein, the key regulator of hypoxia performs the duty of being the prime regulator of cellular and systemic homeostatic responses by the transcriptional activation of many genes under hypoxia and during the heat acclimatization pathway (Bruick and McKnight, 2001; Epstein et al., 2001; Masson et al., 2001; Treinin et al., 2003; Agarwal and Ganesh, 2020). Following long-term heat acclimation, *HIF-1a* is produced at higher levels, and it temporarily upregulates heat shock target genes (Maloyan et al., 2005). According to a molecular study, the encoding open reading frame of *HIF-1a* in bovine species was discovered to be 2469 bp long (Hara et al., 1999). The presence of bHLH, PAS-A, and PAS-B domains was identified and those were reported to have 100 percent similarity to that of *Bos taurus* and *Homo sapiens*. Human composite *HIF-1a* cDNA and encoding ORF have base pair lengths of 3720 bp and 2478 bp respectively (Wang et al., 1995).

In this study, simple techniques such as PCR and bidirectional sequencing were used for the molecular characterisation of the coding regions of the gene. According to Kwok and Chen (2003), direct DNA sequencing or dHPLC (denaturing high-performance liquid chromatography) are the most common methods for finding local target single nucleotide polymorphisms (SNPs). In this study, the number of samples examined was relatively small to confirm the changes observed as SNPs. The variant should be found in at least 1 percent of the population to be classified as SNP. In this work, the detected changes were observed to be nsSNVs (non-synonymous SNVs), which resulted in amino acid differences. Such nsSNVs may exert influence on post-transcriptional modification, binding affinity, folding of the protein, protein expression, and other protein characteristics (Katsonis et al., 2014). The present study must be extended to a larger population to assess whether the changes could be confirmed as SNPs. The functional importance of these variations in deciding the thermo-tolerance and adaptability of these animals need also to be investigated.

Hypoxia contributes significantly to the pathophysiology of most diseases and inflammatory conditions (Duette et al., 2018; Soni and Padwad, 2017; Jin et al., 2020; Zheng et al., 2021). In malignant tissues, a correlation has been observed between HIF-1 overexpression and bad prognosis or resistance to therapy (Semenza, 2003). So the HIF-1 complex was reported to be downregulated in such cases by activating hydroxylases and eventual degradation of HIF-1, for suppression of cancer progression. HIF-1α degradation was accelerated in cells treated with iron and ascorbate by activating the hydroxylases (Knowles et al., 2003). Apart from that *HIF-1a* trans activate genes involved in apoptosis (Eskandani et al., 2017), angiogenesis (Jain et al., 2018), and energy metabolism (Lu et al., 2005). Their protein products boost oxygen supply or promote metabolic hypoxia adaptation. The native Vechur cattle have a marked disease-resistance capacity compared to the cross-bred animals. So this study may form a basis for further evaluating the interrelationships of the observed sequence variations with the disease resistance potential of the animals. This information could be of great value in the selection of heat-resilient animals with better disease-resistance capacity.

5. Conclusions

The full-length cDNA sequences of *HIF-1α* were identified and characterised from Vechur cattle, the native resilient cattle breed of South India. In comparison with similar sequences of bovines and other related species, only two SNVs could be noticed at the 1211th and 2699th positions. The deduced amino acid sequence showed a high level of structural identity with that of the related species except for the change from arginine to lysine at the 311^{th} and 807^{th} positions. The phylogenic tree revealed the highly conserved nature of this protein owing to its importance in homeostatic regulation. The two noted SNVs in the coding regions of the HIF1α gene in Vechur cattle need to be analysed extensively in a larger population along with certain other genes of thermo tolerance in order to establish the correlation of this structural variation to functional resilience. This study would form a base for further functional and genetic studies of this gene in the future.

Declarations

Author contribution statement

Ramya P.R.: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Beena V; Radhika G; Jayavardhanan K.K: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Shynu M: Analyzed and interpreted the data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

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