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Molecular characterization and identification of SNPs in *ATP1A1* isoform of sodium-phosphate adenosine triphosphatase across diverse breeds of riverine buffaloes (*Bubalus bubalis*) and Indian native cattle (*Bos indicus*): A plausible candidate gene for heat tolerance

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ABSTRACT

In the present study, efforts were made to sequence characterize the selected exonic region (18–21) of *ATP1A1* gene to identify variations/SNPs in different breeds of Indian riverine buffaloes and native cattle. The sequence characterization of selected intronic/exonic region (17–21) of *ATP1A1* gene was carried out in a total of 120 samples which included 6 animals each of 8 buffalo breeds and 72 animals of 12 cattle breeds. Genomic DNA was extracted from the whole blood by enzymatic digestion using proteinase K using phenol:chloroform method.. Three sets of primers were designed using Primer3 software to amplify genomic region from intron 17 to intron 21 of *ATP1A1* gene in both cattle and buffaloes. The amplified products were purified by enzymatic method and purified PCR products were sequenced using forward primers in an ABI 3100 Automated DNA Sequencer. The chromatogram of each sequence obtained was checked manually. Base calling was performed with Phred and contig assembly was done via Phrap/Cross_match/Swat tool available in the suite Codon code Aligner v. 3.5.1. The results revealed a total of 26 variations in exons 18–21 of *ATP1A1* gene in riverine buffalo. Out of 26 variations, 6 (T27006876C, C27006599T, T27006345C, T27006330C, G27006309T and T27006240C) were distributed across 4 exonic regions and the remaining 20 were located in intronic region whereas in native cattle, only 2 SNPs were identified in exonic regions (18–21). SNP T27007767C was found to be a novel one in 18 intronic region of *ATP1A1* gene in Indian cattle breeds. All 7 variations found in exonic region of both buffalo and cattle breeds were synonymous with the predicted changes in amino acids. The variations identified in *ATP1A1* gene in the present study could be evaluated in future for their roles in heat tolerance trait in riverine buffaloes and native cattle.

Keywords: *ATP1A1* gene, Heat tolerance, Indigenous cattle, Molecular characterization, Riverine buffaloes, Sequence variations, SNPs

Na^+/K^+-ATPase is a transmembrane protein, member of small family of transporters known as P type ATPase. It is an active ion transporter that maintains the balance of Na^+ and K^+ ions by generating electrochemical gradients across plasma membrane (Blanco *et al*. 1994, Mobasheri *et al*. 2000, Kaplan 2002, Ogawa *et al*. 2009). It is a hetero-oligomer composed of catalytic alpha (α), regulatory beta (β) and gamma (γ) subunits (Jorgensen *et al*. 2003, Sweadner and Rael 2000). The alpha subunit is a major functional unit consisting of ten transmembrane segments (M1 to M10) which enclose the binding site for Na^+ and K^+ ions (Morth *et al*. 2009, Toyoshima *et al*. 2000). It exists in four different isoforms αl, α2, α3 and α4 encoded by *ATP1A1*, *ATP1A2*, *ATP1A3* and *ATP1A4* genes respectively (Shamraj and Lingrel 1994). The *ATP1A1* gene is located on chromosome 3 and comprised 23 exons, with transcript length of 3,152 bps, encoding a protein of 1021 amino acid residues (Liu *et al*. 2010, 2011; Wang *et al*. 2011).

Recently, it has been reported that apart from maintenance of ion transport, Na^+/K^+-ATPase plays an important role in regulating the body temperature and energy transport (Vague *et al*. 2004). An exposure to high temperature results in oxidative stress affecting Na^+/K^+-ATPase activity (Chaiyabutr *et al*. 1997, Li *et al*. 1998, de Lores Arnaiz and Ordieres 2014) that leads to an imbalance in the Na^+ and K^+ ions in dairy animals (Mu *et al*. 2001, Srikandakumar and Johnson 2004). Reports indicated that Na^+/K^+-ATPase activity was positively correlated with heat-resistance trait in Chinese Holstein cattle and reported to have high heritability h~0.53 (Yang *et al*. 2007, Wang *et al*. 2009). Likewise, other studies in Holstein cattle have linked the association of Na^+/K^+-ATPase with thermo-tolerance capacity in dairy animals (Liu *et al*. 2011, Wang

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et al. 2011, Kashyap *et al*. 2014, 2015, Das *et al*. 2015, Deb *et al*. 2015, Kaur *et al*. 2016). Furthermore, various studies reported that polymorphism occurring especially within 17–21 exonic region of Na^+/K^+-ATPase gene, influences physiological functions upon exposure to heat stress, suggesting it as a potential candidate gene for thermotolerance in dairy animals (Liu *et al*. 2010, Liu *et al*. 2011, Wang *et al*. 2011).

Kaur *et al*. (2018a) has sequence characterized the CDS of *ATP1A1* gene in riverine buffaloes (*B. bubalis*) and determined tissue specific expression characteristics of all alpha isoforms using panels of reference genes identified in our previous studies (Kaur *et al*. 2018b). Few attempts have been made earlier to study the polymorphism of Na^+/K^+-ATPase isoforms in Indian cattle (Kashyap *et al*. 2014, Das *et al*. 2015, Kashyap *et al*. 2015) but the studies were limited to either one or fewer breeds.

Although Indian native (zebu) cattle are known to have adapted to heat stress but riverine buffaloes are more prone to thermal stress owing to the presence of morphological and anatomical characteristics with less number of sweat glands, coat colour, etc. Considering the importance of *ATP1A1* gene in heat tolerance it is imperative to explore the genetic structure of *ATP1A1* gene in Indian breeds. The present study was aimed to sequence characterize the genomic region covering the intron 17 to intron 21 of *ATP1A1* gene in 8 breeds of riverine buffaloes and 12 breeds of Indian native cattle.

MATERIALS AND METHODS

Collection of samples: The sequence characterization of selected intronic/exonic region (17–21) of *ATP1A1* gene was carried out in a total of 120 samples. Six animals each of 8 buffalo breeds; Murrah (MUB), Marathwada (MTWB), Mehsana (MHB), Nili Ravi (NRB), Jaffarabadi (JFB), Toda (TDB), South Kanara (SKB) and Kalahandi (KHB) and 72 animals of 12 cattle breeds; Kangayam (KYC), Red Kandhari (RKC), Ladakhi cattle (LAC), Deoni (DEC), Gir (GIC), Kankrej (KJC), Rathi (RAC), Sahiwal (SAC), Tharparkar (THC), Nagori (NAC), Dangi (DAC) and Umblacheri (UMC) from diverse geographical regions of India were included in the study.

DNA isolation and amplification: Genomic DNA was extracted from the whole blood by enzymatic digestion using proteinase K using phenol-chloroform method. The quality and concentration of DNA was estimated by UV-spectrophotometer, and genome DNA was diluted to a final concentration of 50 ng/μL. Three sets of primers (Table 1) were designed using Primer3 software to amplify genomic region from intron 17 to intron 21 of *ATP1A1* gene in both cattle and buffaloes. Each PCR was performed in 25 μL reaction volume containing 200–300 ng genomic DNA, 1X PCR buffer (20 mM Tris-HCl, pH 8.4; 50 mM KCl), 1.5 mM $MgCl_2$, 200 μM dNTPs, 1.0 unit of Taq Polymerase (Invitrogen, CA), and 5 pM of forward and reverse primer. The PCR cycle conditions for the 3 genomic region in both cattle and buffalo are shown in Table 2. After completion of PCR cycles, amplified products were analyzed on ethidium bromide stained 1.5% agarose gel in 1X TAE running buffer (Sigma, USA) and visualized under UV light.

Purification and sequencing of PCR product: The amplified products were purified by enzymatic method using Exonuclease 1 and Antarctic Phosphatase treatment (New England Biolab). The purified PCR products were sequenced using forward primers in an ABI 3100 Automated DNA Sequencer (Applied Biosystems) using Big Dye terminator v 3.1 cycle sequencing kit (ABI, System).

Sequence analysis and haplotypes construction: The chromatogram of each sequence obtained was checked manually. Base calling was performed with Phred (Phred; Codon code Corp.) and contig assembly was done via Phrap/Cross_match/Swat (Phrap; Codoncode Corp.) tool available in the suite Codon code Aligner v. 3.5.1 (CodonCode Corp. Dedham, MA, USA). The nucleotide sequences for different exonic regions of riverine buffalo and Indian native cattle were multiple aligned against representative *B. taurus* reference sequence from GenBank. Contigs were visualized and assembled using molecular evolutionary genetic

Table 1. Primer sequences and PCR conditions for different regions of *ATP1A1* gene

Primer sequence	Amplicon length (bp)	Region amplified	PCR cycle condition cycling temperature		
			Buffalo	Cattle	Time
F: 5′-AGGTTTCTGAGAAGATGGCA-3′	388	Partial intron 17 (83 bp), exon 18	94°C	94°C	60 sec
		(124 bp) and intron 18 (181 bp)	64°C	65°C	60 sec
R: 5′-AGGGAGAGCAAGAATTTGAG-3′			72°C	72°C	60 sec
			30 cycles	30 cycles	
F: 5′-TCTAGAACTTCCGTCCAATG-3′	668	Partial intron 18 (153 bp), exon 19	94°C	94°C	60 sec
		(146 bp), intron 20 (125bp), exon 20	59°C	61°C	60 sec
		(131 bp) and partial intron 20 (113 bp)	72°C	72°C	60 sec
R: 5′-CAAGGAATAGAGGTGGGTGT-3			30 cycles	30 cycles	
F: 5′-TGCTGATTCCTGGTCTGCAT-3′	455	Partial intron 20 (182 bp), exon 21 (102 bp)	94°C	94°C	60 sec
R: 5′-CTGGTCAGGGAGCTACATGC-3′		and partial intron 21 (171 bp)	65°C	67°C	60 sec
			72°C	72°C	60 sec
			30 cycles	30 cycles	

Table 2. Frequency of identified variations in the exonic region across different Indian buffalo breeds

Region	Nucleotide position	Genotype	Genotype frequency									Allele frequency (Overall)	
			MU	MTW	MH	NR	JF	TD	SK	KH	Overall		
Exon-18	T27006876C	TT	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.125	T	C
		CC	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	0.875	0.125	0.875
Exon-19	C27006599T	TT	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	C	T
												0.00	1.00
Exon-20	T27006345C	CC	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	T	C
												0.00	1.00
Exon-20	T27006330C	CC	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	T	C
												0.00	1.00
Exon-20	G27006309T	GT	0.33	0.00	0.67	0.00	0.00	0.33	0.00	0.00	0.17	G	T
		TT	0.67	1.00	0.33	1.00	1.00	0.67	1.00	1.00	0.83	0.08	0.92
Exon-20	T27006240C	CC	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	T	C
												0.00	1.00

analysis (MEGA) software version 6.0 using Clustal W (Tamura *et al*. 2011). The allele frequency of SNPs identified in different regions of *ATP1A1* was determined by dividing the number of times the allele of interest was observed in a population divided by the total number of copies of all the alleles at that particular genetic locus in the population. Haplotypic variations in the *ATP1A1* gene were estimated using accelerated EM algorithm similar to the partition/ligation method creating highly accurate population frequency estimates of the phased haplotypes based on the maximum likelihood as determined from the unphased input. Linkage disequilibrium (LD) was estimated using HAPLOVIEW.

RESULTS AND DISCUSSION

In the present study, genomic region spanning between partial intron 17 to partial intron 21 of *ATP1A1* gene was sequence characterized in 8 breeds of Indian riverine buffaloes and 12 breeds of Indian native cattle. Amplified PCR products with the length of 388 bp, 668 bp, and 455 bp targeted the regions of partial intron 17-partial-intron 18; partial intron 18-partial intron 20; and partial intron 20-partial intron 21, respectively (Fig. 1A, B, C). The sequence alignment of both buffalo and cattle were retrieved in form of chromatograms (Fig. 2). Using Codon code Aligner v. 3.5.1, the sequences of riverine buffalo breeds were aligned and compared with exotic cattle. Sequence data revealed a total of 26 variations in exonic regions spanning 18–21 exons. Out of 26 variations in riverine buffalo, 6 variations were distributed across 4 exonic regions (T27006876C C27006599T, T27006345C, T27006330C, G27006309T and T27006240C respectively) and the remaining 20 were found in intronic region. On the other hand, only 2 variations were found in cattle breeds in the selected genomic regions of *ATP1A1* gene. Out of two SNPs, one SNP (C2544A/C27007790A) was identified in the exonic region while the other (T27007767C) in intronic region. All 7 exonic variations in both riverine buffalo and cattle breeds were synonymous in nature.

Analysis of partial genomic region spanning between intron 17 to intron 18 of ATP1A1: Multiple sequence alignment of the buffalo sequence data with *Bos taurus* reference sequence (accession number: NM_001076798.1) spanning genomic regions covering partial intron 17, exon 18 and intron 18, revealed a total of 8 novel variations with one variation in exon 18 (T2458C/T27006876C), while rest were present in intronic region (A27006922G, T27006919C, C27007733A, T27007728A, G27007654C, A27007651G and G27007650T). Along with these variations, a deletion of nucleotide "G" at position number 27007731 and an insertion of 3 bases "AAC" were also observed. Allele and genotypic frequency of all variations are given in Tables 3 and 4. Among variations, all were completely fixed with a frequency of 1.00 in Indian buffalo

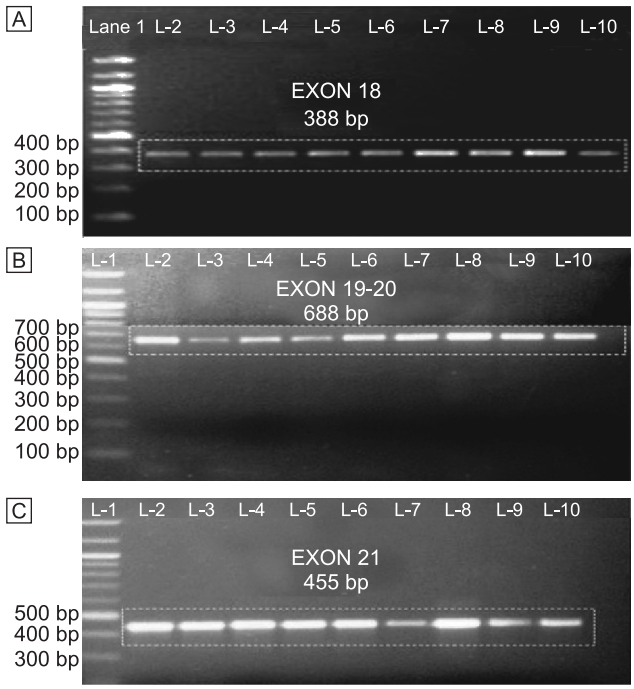

Fig. 1. 1.5% agarose gel showing amplified products of different region of *ATP1A1* gene (Lane 1: 100 bp Ladder, Lane 2–6: Representatives of riverine buffalo breeds; Lane 7–10: Representatives of Indian cattle breeds).

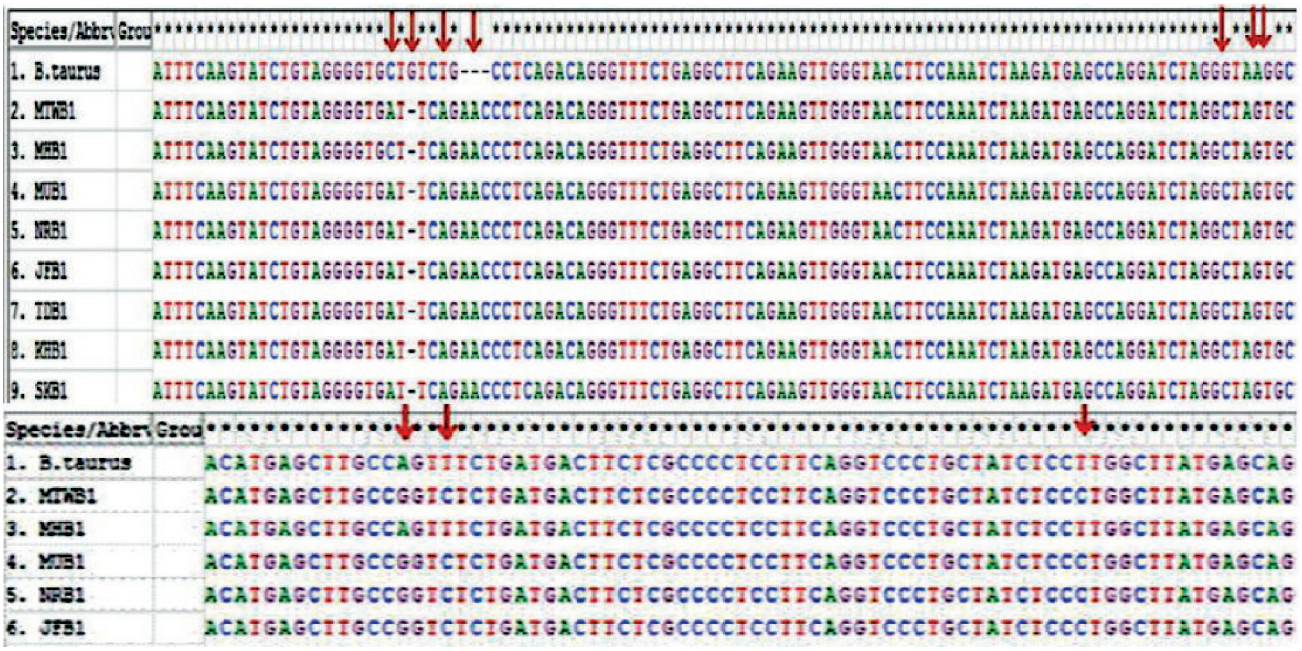

Fig. 2. Sequence alignment of exonic region 18 of *ATP1A1* gene in different buffalo breeds with *Bos taurus*.

Fig. 3. Sequence alignment of exonic region 18 of *ATP1A1* gene in different cattle breeds with *Bos taurus*.

breeds, except A27006922G, T27006919C and T27006876C (Tables 3, 4).

Multiple sequence alignment of sequence from Indian native cattle breeds against the *Bos taurus* (NM_001076798.1) showed only 2 variations; one in exon 18 (C2544A/C27007790A) and other in intron 18 (T27007767C). The second SNP identified at cDNA position 353 (T27007767C) in intron 18 of *ATP1A1* gene in Indian cattle breeds was identified as a novel variation, since it was neither reported in any literature nor available

Table 3. Frequency of identified variations in the intronic region across different Indian buffalo breeds

Region	Nucleotide position	Genotype	Genotype frequency									Allele frequency (Overall)	
			MU	MTW	MH	NR	JF	TD	SK	KH	Overall		
Intron-17	A27006922G	AA	0.00	0.00	1.00	0	0	0	0	0	0.125	A	G
		GG	1.00	1.00	0	1.00	1.00	1.00	1.00	1.00	0.875	0.125	0.875
Intron-17	T27006919C	TT	0.00	0.00	1.00	0	0	0	0	1.00	0.25	T	C
		CC	1.00	1.00	0	1.00	1.00	1.00	1.00	0	0.75	0.25	0.75
Intron-18	C27007733A	CC	0.00	0.00	1.00	0	0	0	0	0	0.125	C	A
		AA	1.00	1.00	0	1.00	1.00	1.00	1.00	1.00	0.875	0.125	0.875
Intron-18	T27007728A	AA	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	T	A
												0	1.00
Intron-18	G27007654C	CC	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	G	C
												0	1.00
Intron-18	A27007651G	GG	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	A	G
												0	1.00
Intron-18	G27007650T	TT	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	G	T
												0	1.00
Intron-19	G27006407T	GG	0.67	1.00	1.00	1.00	1.00	0.33	1.00	1.00	0.875	G	T
		GT	0.33	0.00	0.00	0	0	0.67	0	0	0.125	0.94	0.06
Intron-20	C27006191T	CC	0	0.00	0.67	0	0	0	1.00	0	0.21	C	T
		CT	0.33	0.00	0.33	0	0	0	0	0	0.08		
		TT	0.67	1.00	0	1.00	1.00	1.00	0	1.00	0.71	0.25	0.75
Intron-20	G27006168A	AA	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	G	A
												0	1.00
Intron-20	A27005882G	AA	0.67	0.33	0.67	1.00	1.00	0	0	1.00	0.58	A	G
		AG	0.33	0.67	0.33	0	0	0.33	0.67	0	0.29		
		GG	0	0	0	0	0	0.67	0.33	0	0.13	0.73	0.27
Intron-20	A27005825G	AG	0.33	0.67	0.33	0	0	0.33	0.67	0	0.29	A	G
		GG	0.67	0.33	0.67	1.00	1.00	0.67	0.33	1.00	0.71	0.15	0.85
Intron-20	C27005820G	GG	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	C	G
												0	1.00
Intron-20	G27005813A	AA	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	G	A
												0	1.00
Intron-20	A27005778G	AA	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	0.96	A	G
		AG	0	0	0	0.33	0	0	0	0	0.04	0.98	0.02
Intron-20	T27005742C	CC	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	T	C
												0	1.00
Intron-21	T27005620C	TT	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	T	C
												0	1.00
Intron-21	C27005587T	CC	0.67	0.33	0.67	1.00	1.00	0.33	1.00	1.00	0.75	C	T
		CT	0.33	0.67	0.33	0	0	0.67	0	0	0.25	0.875	0.125
Intron-21	C27005569A	CC	0.67	0.33	0.67	1.00	1.00	0.33	1.00	1.00	0.75	C	A
		AC	0.33	0.67	0.33	0	0	0.67	0	0	0.25	0.875	0.125
Intron-21	T27005544A	AA	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	T	A
												0	1.00

in database. For both variations located at C27007790A and T27007767C, heterozygous genotypes were observed in Indian cattle breeds (Fig. 4; Table 5).

Analysis of partial genomic region spanning intron 18 to intron 20 of ATP1A1: Sequence data of 668 bp covering intron 18, exon 19, intron 20, exon 20 and partial intron 20 for both buffalo and cattle breeds is shown in supplementary Fig. 5. The sequence data of riverine buffaloes revealed a total of 8 variations when compared with taurine cattle. Out of which, 5 variations were found in the exon 19 and exon 20 (C27006599T, T27006345C, T27006330C, G27006309T and T27006240C) and remaining three were found in the intronic region (G27006407T, C27006191T and G27006168A). Heterozygous condition was observed at 3 sites (G27006407T, G27006309T and C27006191T) (Fig. 6 (A-B); Tables 3, 4). However, no variation was observed in Indian native cattle breeds in comparison to exotic cattle (*Bos taurus*) (Fig. 7).

Analysis of partial genomic region spanning intron 20 to intron 21 of ATP1A1: Sequence data of 455 bp covering partial region of intron 20, exon 21 and partial intron 21, revealed a total of 10 novel SNPs (A27005882G, A27005825G, C27005820G, G27005813A, A27005778G, T27005742C, T27005620C, C27005587T, C27005569A and T270005544A) in intronic region of bubaline *ATP1A1* gene (Fig. 8; Tables 3, 4). The heterozygous genotypes were

Table 4. Frequency of identified variation in the exonic region across different Indian cattle breeds

Nucleotide position	Genotype	Genotype frequency												Allele frequency (Overall)	
		SW	TH	RT	KY	RK	LA	DE	GR	KJ	DG	NG	UMC	Overall	
C27007790A	CC	0.67	0.67	1	1	1	1	1	1	1	1	0.67	0.67	0.89	C A
(Exon-18)	CA	0.33	0.33	0	0	0	0	0	0	0	0	0.33	0.33	0.11	0.94 0.06
T27007767C	TT	0.67	0.33	0.67	1	0	1	0	1	1	0.67	0.67	0.67	0.64	T C
(Intron-18)	TC	0	0.34	0.33	0	0.33	0	1	0	0	0	0	0.33	0.19	0.74 0.26
	CC	0.33	0.33	0	0	0.67	0	0	0	0	0.33	0.33	0	0.17	

Fig. 4. Sequence alignment of exonic region 19–20 of *ATP1A1* gene in different buffalo breeds with *Bos taurus*.

observed at 5 positions (A27005882G, A27005825G, A27005778G, C27005587T and C27005569A) (Fig. 9 (A-B; Tables 3, 4).

A total of 6 SNPs in exonic regions were identified in riverine buffalo compared with *Bos taurus* cattle. SNP G27006309T present in exon-20 was found to be polymorphic with 2 genotypes (GT and TT) as observed in Murrah, Mehsana and Toda buffaloes. Rest all 5 loci were found monomorphic in all the buffalo breeds. In intronic regions, a total of 20 SNPs were identified. Whereas, only 7 loci (G27006407T, C27006191T, A27005882G, A27005825G, A27005778G, C27005587T and C27005569A) were found polymorphic in different buffalo breeds. However, no variation was observed in Indian cattle breeds aligned against the exotic cattle (*Bos taurus*) (Fig. 10).

Haplotype and linkage disequilibrium analysis: Overall, 12 SNPs were observed in the *ATP1A1* gene in the selected exonic regions. Haplotypes were estimated using accelerated EM algorithm (Excoffier and Slatkin 1995) and by applying the 4 gamete rule and estimates of recombination revealed 2 blocks in buffalo *ATP1A1* gene both of which showed strong LD. The LD plot with haplotypes along with their frequencies is shown in Fig. 11. Block 1 showed 2 SNPs belonging to exon 20 and intron 20 whereas block 2 had SNPs from intronic region 21.

Na^+/K^+-ATPase has a significant role in different physiological traits as ubiquitous expression of *ATP1A1* gene suggests its role in maintaining cellular Na^+ and K^+ homeostasis in all the cells and tissues. The present study identified the polymorphic sites in heat stress associated regions in cattle and buffalo the 2 important dairy species

Fig. 5. Sequence alignment of exonic region 21 of *ATP1A1* gene in different buffalo breeds with *Bos taurus*.

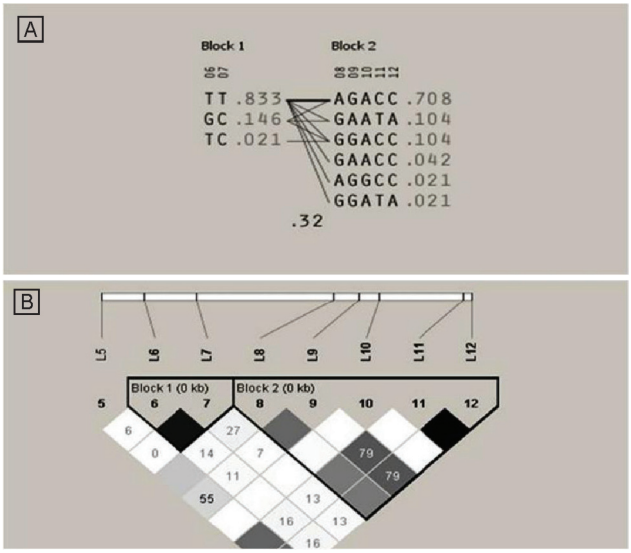

Fig. 6. Pair-wise measure of LD relationship among the variations identified for *ATP1A1* gene—(A) 2 haploblocks were identified, (B) haplotypes for the variations (black colour).

of India. The variations were identified in studied regions were mostly conserved in Indian cattle breeds. It is interesting that most of the identified SNPs were reported earlier and have association with heat stress tolerance in cattle. Genetic polymorphism of the alpha 1 isoform of Na^+/K^+-ATPase (*ATP1A1*) (Liu *et al*. 2010, 2011) and Na^+/K^+-ATPase β2 subunit (*ATP1B2*) (Wang *et al*. 2011) has been reported in Chinese Holstein suggesting Na^+/K^+-ATPase (NKA) activity favoured with anti-heat stress traits. The novel SNP identified in exon 18 (T2458C/T27006876C) would be interesting to explore more with disease or stress state in cattle breeds. Several studies associated the polymorphism of *ATP1A1* with heat tolerance traits and so this gene may be adopted as DNA marker in dairy cattle. The SNP C27007790A identified in present study was significantly associated with respiration rate under heat stressed state in Jersey crossbred cows (Das *et al*. 2015). The nucleotide substitution at cDNA position 2544 from C to A (rs110256520; Chromosome 3:27007790) found in present study has been reported by other workers in Chinese Holstein cows (Liu *et al*. 2011); zebu-taurine crossbreds (Vrindavani) and indigenous zebu cattle (Tharparkar) (Kashyap *et al*. 2015); Jersey crossbred cows (Das *et al*. 2015). These studies have indicated a higher level of mRNA *ATP1A1* as temperature increases indicating that it may be promising as a potential candidate for anti-heat stress. Similarly, Liu *et al*. (2010) reported 2 SNPs in P14 locus: G14103A and C14242T in exon 14 and intron 14 of bovine *ATP1A1* gene, respectively, both of which indicated that the polymorphism site has contributed for anti-heat stress trait. Likewise, few attempts have been made to study the polymorphism of Na^+/K^+-ATPase isoforms in Indian cattle (Kashyap *et al*. 2014, Das *et al*. 2015, Kashyap *et al*. 2015); in Jersey crossbred cows (Das *et al*. 2015) which also signified the role of Na^+/K^+-ATPase against heat stress.

Expression pattern of *ATPase* beta subunit genes (*ATPase B1*, *ATPase B2* and *ATPase B3*) among the crossbred bulls was analysed under different temperatures (20–44°C) by Deb *et al*. (2015) which showed a significant increase in the transcript expression of all beta isoforms under thermal stress. Among various potential candidates genes for heat tolerance traits in dairy animals, Na+/K+-ATPase seems to be promising as a potential DNA marker for thermotolerance in dairy animals. Recently, it is reported that Na+/K+ATPase regulates bovine sperm capacitation through a protein kinases A and C (Thundathil *et al*. 2006). In addition, this ATPase enzyme plays a role in the mechanism of cell death and apoptosis (Yu 2003), which is associated with various disease states. The polymorphism at exon-17 of bovine ATP1A1 has been associated with altered activity across red cell membrane and somatic cell score in cattle and reported that mastitis resistant animals have high Na^+ (/)K^+ ATPase activity (Liu *et al*. 2012). Na^+/K^+ ATPase is responsible for maintenance of membrane potential and in conjugation with other cofactors, ultimately result in the transepithelial movement of water from blood to the lumen of the sweat gland (i.e. sweat production) (Sato and Sato 1989). Previous studies have shown that the *ATP1A1* gene polymorphism influences the Na^+-K^+-ATPase activity in C-peptide deficiency (Jannot *et al*. 2002). Altered activity of Na^+-K^+-ATPase results in changes in ion concentration of K^+ and Na^+ that might affect the osmolality of milk and feeding of high osmolality milk could cause necrotizing enterocolitis in infants (Pearson *et al*. 2013).

Since it is an important regulator of homeostasis, characterisation of Na^+/K^+-ATPase is imperative in ruminant species. Few efforts have been made in porcine, showed high polymorphic nature of this ATPase gene (Larsen *et al*. 2019, Henrisken *et al*. 2013). In porcine, α subunit (*ATP1A1*, *ATP1A2* and *ATP1A3*) was sequenced and reported at 5 different SNPs in α-1 subunit (Henriksen *et al*. 2013). To the best of our knowledge, the present study is the first to report these polymorphic sites in Na^+-K^+-ATPase in Indian buffalo (riverine) breeds. A set of novel SNPs were identified in buffalo breeds and would be interesting to study their effect on its activity.

As stated above that polymorphic sites affect the traits of dairy animal, hence the polymorphism site described in this study may be a used as a potential molecular marker to study the heat tolerance/resistance traits in dairy animals, although further studies are needed to be carried on larger population to expand its application.

In conclusion, the present study helped to enrich the bubaline and indicine genomic data with respect to *ATP1A1* gene. The identified variations identified in *ATP1A1* gene could be evaluated in future for their roles in heat tolerance trait in riverine buffaloes and cattle, as this gene is known to play significant role in heat stress response.

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