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Single nucleotide polymorphisms in ATP1A1 gene and their association with thermotolerance traits in Sahiwal and Karan Fries cattle

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ABSTRACT

Expression of stress genes under thermal stress partially explain the relatively higher thermal adaptability of indigenous breeds compared to exotic breeds of cattle. ATP1A1 gene is one of such gene that encodes for α1 isomer of Na^+, K^+-ATPase enzyme for Na^+- coupled transport of metabolites, nutrients, ions and represents a plausible candidate for heat tolerance traits. Present study was designed to compare SNP variations in ATP1A1 gene and to evaluate their association with respiration rate, rectal temperature and heat tolerance coefficient in Sahiwal (51) and Karan Fries (50) cows maintained at Livestock Research Centre of National Dairy Research Institute, Karnal. Two SNPs (T27008243C and A27008223G) were identified in both the dairy cattle breeds. Association of each SNP genotype was analyzed using Generalized Linear Model procedure in Statistical Analysis System (SAS). Sahiwal cows with TT (15.91 ± 1.89^{b}) at T27008243C locus had lowest RR compared to TC genotypes (18.25 ± 1.77^{ab}) and CC genotype (19.24 ± 1.52^{a}), while in Karan Fries cows RR for AA genotype at A27008223G locus was lower (28.85 ± 1.96^{b}) compared to GG genotype (32.37 ± 2.51^{a}). Thus, the study indicated that the TT genotype at T27008243C locus in Sahiwal and AA genotype at 27008223 locus in Karan Fries cows are desirable genotypes for genetic adaptability under heat stress.

Key words: ATP1A1, Heat tolerance coefficient , Rectal temperature, Respiration rate, SNPs.

INTRODUCTION

Heat stress resulted from high ambient temperature and humidity has huge economic impact on the global dairy industry, and has adverse effects on health, production and reproduction in dairy animals. Heat stress also causes oxidative stress in dairy cows (Bernabucci *et al*., 2002) and influences the plasma electrolyte balance (Banerjee and Ashutosh, 2011) with a significant change in Sodium-Potassium ATPase enzyme (Na^+, K^+-ATPase) activity (Kashyap *et al*., 2014). This transmembrane carrier enzyme is responsible for establishing electrochemical gradient of Na^+ and K^+ ions across the plasma membrane and represents a plausible candidate for heat tolerance traits (Geering *et al*., 1987). The ATP1A1 gene coding for α1 isomer of Na^+, K^+- ATPase enzyme expressed ubiquitously in almost all the tissues and is essential for cell viability, though the expression level varies among different tissues (Kaplan, 2002). The α1-subunit gene (ATP1A1) is a member of the housekeeping genes and presents a plausible candidate responsible for heat tolerance traits. ATP1A1 gene is located on *Bos taurus* autosome 3 spanning total length of 22768 base pair with mRNA length of 3746 bases and has coding sequence of 3066 nucleotides (http://www.ensembl.org).

MATERIALS AND METHODS

The experimental design and procedure were carefully planned and approved by the Institute's Animal Ethical Committee.

Blood sample were collected randomly from 51 Sahiwal cows and 50 Karan Fries cows maintained at Livestock Research Centre, National Dairy Research Institute, Karnal. Genomic DNA was extracted from the frozen/thawed blood samples using phenol-chloroform extraction method as described by Sambrook *et al*. (1989) with minor modifications. Quality of genomic DNA was checked on 0.8% agarose gel electrophoresis while its quantification was done using Biospec-nano spectrophotometer method.

Measurement of physiological parameters: Two physiological parameters namely respiration rate (RR) and rectal temperature (RT) were recorded three times consecutive and average was taken as final reading for association analysis for all the animals. The data were recorded at the probable extreme hours of day i.e. 6-8 am, 12-2 pm and 12-2 pm during winter (Jan), spring (Mar) and summer (June) seasons respectively. Heat tolerance coefficient (HTC) was also determined based on heat

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tolerance index developed by Benezra (1954) with the following equation:

HTC (*Benezra Coefficient of Heat Adaptability*) = *RR/23 + RT/38.33*

In the equation, the denominator 23 and 38.33 are normal RR and RT of cattle under ideal conditions. According to Benezra (1954) lower the value determined by the equation higher the degree of adaptability.

The outdoor temperature and the relative humidity (RH%) were recorded daily during the experiment to ascertain Temperature humidity index (THI) value and were used in the association analysis study as fixed variables. THI was calculated according to formula developed by National Research Council (NRC, 1971):

$$THI = 0.72 (Wb + Db) + 40.6$$

Where, Wb is wet bulb temperature and Db is dry bulb temperature in °C

The value for THI were lowest for winter (49.70), followed by spring (64.65) and highest for summer (86.44), thus spring was considered as reference season for the present study.

PCR amplification of Bovine ATP1A1 Gene and detection of polymorphism : The primers used in this experiment are based on earlier report by Das *et al.*, (2015). The PCR reactions were carried out in a total of 25 µl volume containing 3 µl genomic DNA (50 ng/ µl), 0.5 µl of each forward and reverse primer, PCR Master Mix (2X) (Fermentas) of 12.5 µl and 8.5 µl of water. Amplification was performed in a Thermal cycler (BioRad T100). The thermal cycling conditions involved an initial denaturation at 95°C for 3 min, followed by 34 cycles with denaturation at 94°C for 30 sec, annealing temperature of 56.5°C for 30 sec and extension at 72°C for 25 sec. The reaction was terminated after a final extension at 72°C for 8 min. The confirmation of each PCR amplification products of desired target was done using 2% agarose gel electrophoresis stained with 1% ethidium bromide. The amplified PCR product was purified and custom sequencing from both ends (5' and 3' ends) by outsourcing (Merck Specialties Pvt. Ltd. Bangalore). The raw sequence was analyzed and further ClustalW multiple sequence alignment program was used to align respective sequence with reported *Bos taurus* sequence (ENSBTAG00000001246) to detect any nucleotide(s) changes.

Statistical analysis: Allele and genotype frequencies were calculated using POPGENE software package (Yeh *et al.*, 1999). Significant relationship of each SNP locus with RR and RT in Sahiwal and Karan Fries cows was analyzed using Generalized Linear Model (GLM) in Statistical Analysis System (SAS). The significant effect of SNP variants on physiological traits were analyzed using the following model:

$$Y_{ijkl} = \mu + P_i + T_j + G_k + G_l + e_{ijkl}$$

Where,

Y_{ijkl} = Y_{ijkl} is the l^{th} observation on RR/RT/HTC of cows in i^{th} Parity, j^{th} THI, k^{th} genotype and l^{th} genotype

μ = Overall mean

P_i = Effect of i^{th} parity

T_j = Effect of j^{th} THI

G_k = Fixed effect of k^{th} genotype

G_l = Fixed effect of l^{th} genotype

e_{ijkl} = Random error associated with Y_{ijkl} observation and assumed to be NID (0, $\sigma^2 e$)

RESULTS AND DISCUSSION

The range of average values of RR, RT and HTC during winter, spring and summer seasons showed that RR was highest (40/minute) during summer and lowest (10/minute) during winter in Sahiwal cows, while, it was highest (70/minute) during summer and lowest (9/minute) during winter in Karan Fries cows. The RT was highest (102.7°C) during summer and lowest (94.5°C) during winter in Sahiwal cows while it was highest (104.6°C) during summer and lowest (96.1°C) during winter in Karan Fries cows. Similar trends were also observed in case of HTC where, highest (2.76) during summer and lowest (1.33) during winter in Sahiwal cows while it was highest (4.10) during summer and lowest (1.32) during winter in Karan Fries cows.

In this study, 491 bp fragments covering partial exon 16 to partial intron 17 of bovine ATP1A1 gene (chr. 3: 27,002,873-27,025,641) were targeted based on study reported earlier by Das *et al.*, (2015). Targeted regions of ATP1A1 gene was successfully PCR amplified for each animal of both Sahiwal and Karan Fries breed. The custom sequencing and multiple alignments revealed one new SNP (T27008243C) and one reported (Das *et al.*, 2015) SNP (A27008223G) in the intronic regions in Sahiwal (Fig. 1) and Karan Fries breeds (Fig. 2). The allelic and genotypic frequencies of ATP1A1 gene are given in Table 1. At SNP

Table 1: Allelic and Genotypic Frequency at each SNP locus of ATP1A1 gene in Sahiwal and Karan Fries cows

SNP	Sahiwal					Karan Fries			
	Allelic Frequency		Genotypic Frequency			Allelic Frequency		Genotypic Frequency	
T27008243C	T	C	TT	TC	CC	T	C	TT	TC
	0.39	0.61	0.26	0.27	0.47	0.81	0.19	0.62	0.32
A27008223G	A	G	AA	AG	GG	A	G	AA	GG
	0.12	0.88	0.08	0.08	0.84	0.78	0.22	0.78	0.22

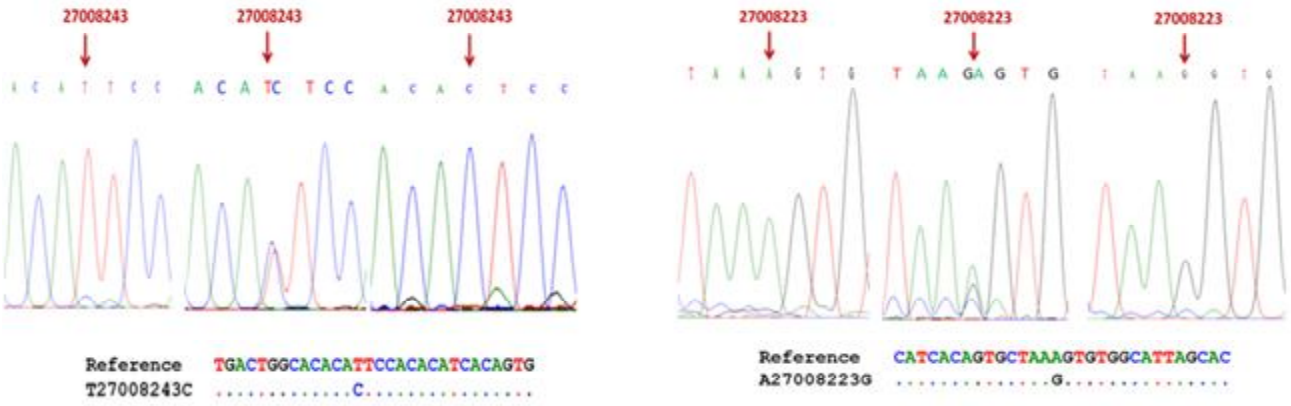

Fig 1: DNA sequence showing changes of T27008243C and A27008223G of ATP1A1 gene in Sahiwal cows

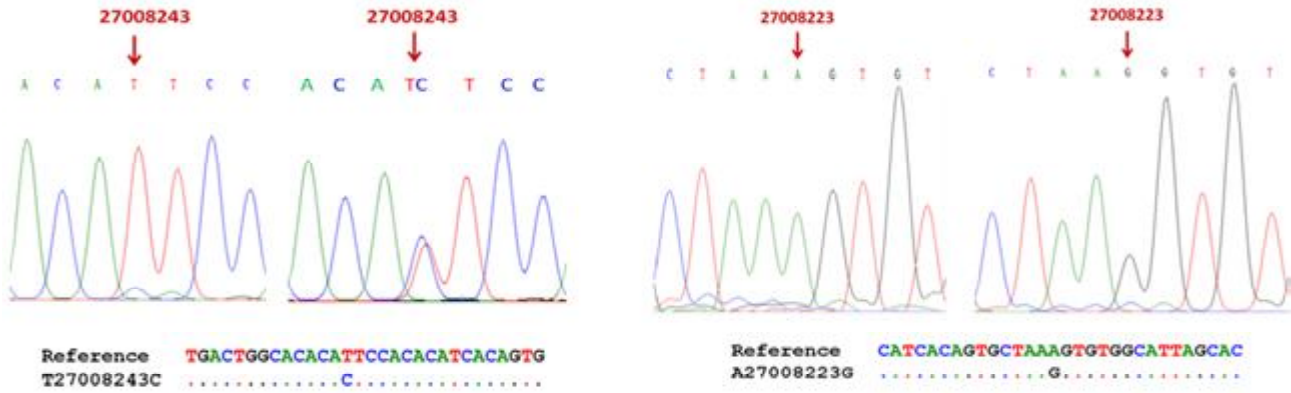

Fig 2: DNA sequence showing changes of T27008243C and A27008223G of ATP1A1 gene in Karan Fries cows

locus T27008243C, it was observed that allelic frequency of allele 'C' was highest (0.61) in Sahiwal and frequency of allele 'T' was highest (0.81) in Karan Fries cows. Similar pattern was observed at SNP locus A27008223G where allelic frequency of allele 'G' was highest (0.88) in Sahiwal and frequency of allele 'A' was highest (0.78) for Karan Fries cows.

Heat tolerance traits such as RR, RT and HTC of both Sahiwal (Table 2) and Karan Fries cows (Table 3) differed highly significantly ($p<0.001$) in all three THI subclasses of different seasons. Significant ($p<0.05$) association of SNP locus T27008243C were observed for RR with genotype TT having lower RR (15.91 ± 1.89^{b}) compared to TC (18.25 ± 1.77^{ab}) and CC (19.24 ± 1.52^{a}) (Table 2), while in case of Karan Fries cows, significant ($p<0.05$) association of SNP with RR at locus A27008223G was observed with genotype AA (28.85 ± 1.96^{b}) having lower RR compared to genotype GG (32.37 ± 2.51^{a}) (Table 3). RT and HTC did not show any association with SNP genotypes in both the breeds

Among various physiological reactions of animal studied under heat stress RR and RT were observed as most sensitive indices (Verma *et al*., 2000). Rectal temperature was recognized as an important measure of physiological status in heat stressed animals as even a rise of less than 1°C in rectal temperature was enough to reduce performance in most livestock species (McDowell, 1976). In the present study, the observed pattern in RR, RT and HTC revealed that with increase in THI level there was increased in all of these thermoregulatory responses by the cows, as previously mentioned by Das *et al*. (2015) while observing Jersey crossbred cows under different THI levels.

Multiple cellular mechanisms are displayed to neutralize the heat stress impacts. One of the cellular responses includes altered expression of stress genes such as "heat shock proteins" (HSP27, HSP60, HSP70, HSP90 and HSP110) under thermal stress (Collier *et al*., 2008). Moreover, approximately 50 genes including ATP1A1 gene not traditionally considered as HSPs but shows altered expression to adapt under heat stress (Sonna *et al*., 2002). Identification of SNPs in these stress responsible genes and their association with variation in animal sensitivity to thermal stress will permit screening of animals having presence or absence of desirable or undesirable alleles (Collier *et al*., 2008). Na^{+}, K^{+}- ATPase α1-subunit gene (ATP1A1) polymorphisms were associated with heat

Table 2: Least squares means of subclasses of different fixed effects for RR, RT and HTC in Sahiwal cows

Effect	Subclass	RR	RT	HTC
Overall Mean		20.02±0.60	38.08±0.06	1.90±0.03
Seasons (THI)	Winter (THI=49.70)	12.78±1.64^{c}	37.30±0.17^{c}	1.53±0.06^{c}
	Spring (THI=64.65)	15.09±1.64^{b}	38.17±0.17^{b}	1.64±0.06^{b}
	Summer (THI=86.44)	25.55±1.64^{a}	38.79±0.17^{a}	2.20±0.06^{a}
Parity	1 (01)	25.70±3.32	38.23±0.34	2.10±0.13
	2 (09)	17.15±2.22	38.00±0.23	1.71±0.08
	3 (10)	18.13±1.91	38.12±0.20	1.73±0.07
	4 (07)	17.86±1.97	38.08±0.20	1.71±0.07
	5 (11)	18.75±1.82	38.19±0.19	1.74±0.07
	6 (05)	17.53±2.06	38.05±0.21	1.81±0.08
	7 (07)	16.81±1.80	37.97±0.18	1.79±0.07
	9 (01)	10.50±3.35	38.05±0.34	1.79±0.13
T27008243C	TT (13)	15.91±1.89^{b}	38.08±0.19	1.74±0.07
	TC (14)	18.26±1.77^{ab}	37.99±0.18	1.82±0.07
	CC (24)	19.24±1.52^{a}	38.18±0.16	1.84±0.06
A27008223G	AA (04)	16.90±2.01	38.08±0.21	1.74±0.08
	AG (04)	18.29±2.23	38.16±0.23	1.83±0.08
	GG (43)	18.22±1.51	38.01±0.16	1.82±0.06

Figures in parenthesis are number of animals;
Figures with dissimilar superscript differ significantly

Table 3: Least squares means of subclasses of different fixed effects for RR, RT and HTC in Karan Fries cows

Effect	Subclass	RR	RT	HTC
Overall Mean		29.13±1.21	38.21±0.07	1.90±0.03
Seasons (THI)	Winter (THI=49.70)	17.70±2.23^{c}	37.07±0.22^{c}	1.70±0.07^{c}
	Spring (THI=64.65)	24.90±2.23^{b}	37.98±0.22^{b}	1.82±0.07^{b}
	Summer (THI=86.44)	49.22±2.23^{a}	38.77±0.22^{a}	2.35±0.07^{a}
Parity	2 (07)	33.59±2.14^{a}	37.96±0.21	1.91±0.06
	3 (17)	32.95±2.49^{a}	37.83±0.24	1.92±0.07
	4 (12)	30.27±2.07^{a}	37.85±0.20	1.98±0.06
	5 (08)	32.74±2.48^{a}	37.86±0.24	1.96±0.07
	6 (04)	32.26±2.78^{a}	37.98±0.27	1.99±0.08
	7 (02)	21.82±3.28^{b}	38.17±0.32	1.97±0.10
T27008243C	TT (31)	30.89±2.39	37.90±0.23	1.93±0.07
	TC (19)	30.32±2.03	37.98±00.20	1.98±0.06
A27008223G	AA (39)	28.85±1.96^{b}	37.88±0.19	1.93±0.06
	GG (11)	32.37 ±2.51^{a}	38.00±0.24	1.98±0.07

Figures in parenthesis are number of animals;
Figures with dissimilar superscript differ significantly

tolerance in Chinese Holstein cattle (Liu *et al*., 2010; Liu *et al*., 2011), Murrah Buffalo (Jayakumar, 2014), Jersey crossbred cows (Das *et al*., 2015) and in Tharparkar and Vrindavani crossbred cattle (Kashyap, 2015). In our study, all possible three genotypes (homozygous and heterozygous) at each SNP locus were present in Sahiwal cows. However, in Karan Fries cow's genotype CC at locus T27008243C and genotype AG at locus A27008223G was not observed. Our study corresponds to study conducted by Das *et al*. (2015) in Jersey crossbred cows where they did not found genotype AG at locus A27008223G. Moreover, SNP locus T27008243C were also not observed in their study. Sahiwal cows of TT genotype at T27008243C locus and Karan Fries cows of genotype AA at SNP locus A27008223G has least respirations per minute and were regarded as better thermotolerant compared to remaining cows. Charoensook *et al*. (2012) reported a low RR may indicate an improved thermotolerance adept by animals.

CONCLUSION

Present study revealed that Sahiwal cows with TT genotype at T27008243C locus and Karan Fries cows with genotype AA at SNP locus A27008223G were better thermotolerant compared to remaining cows. It is suggested that the bovine ATP1A1 gene may play an important role in heat resistance in dairy cows and the SNP identified could be a potential useful genetic marker for aid to selection for

anti-heat stress traits in dairy cattle breeding programmes. However, large number of samples from different genetic groups of animals is needed before arriving at a concrete conclusion regarding thermoregulatory role of ATP1A1 gene.

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