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Gene substitution effect of bovine *heat shock protein beta-1* gene polymorphism on age at calving in Indian dairy cattle

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ABSTRACT

The study was planned with objective to screen single nucleotide polymorphisms (SNPs) in bovine *HSPB1* gene and to find its effect on age at calving in Karan-Fries (*Bos taurus* × *Bos indicus*) and Sahiwal (*Bos indicus*) breeds of cattle. Genomic DNA was extracted from whole blood of 180 cows of both breeds. Based on publically available bovine *HSPB1* gene sequence, one primer set was used for polymerase chain reaction amplification of the target region. Further DNA sequencing revealed a transition of thymine to cytosine at SNP rs208395876 in 5'UTR and a silent transversion of guanine to thymine at SNP rs723061520 in first coding sequence of bovine *HSPB1* gene in both the studied breeds. Effect of individual SNP genotypes of bovine *HSPB1* gene with age at calving (months) was analyzed separately in both breeds via regression using a repeated gene substitution MIXED model and least-squares means.The overall observed heterozygosity in both breeds and the F-Statistics values indicated that there was lesser genetic diversity in studied genomic region of bovine *HSPB1* gene in *Bos indicus* compared to crossbred cattle. Association analysis revealed that SNP rs208395876 significantly delayed age at calving in Karan-Fries cows. In conclusion, the studied genomic region of bovine *HSPB1* gene is polymorphic. In addition, these polymorphisms were informative with regard to age at calving of crossbred cows. Therefore, this gene is an important candidate for cow fertility.

Key words: Age at calving, *Bos indicus*, Gene substitution model, *HSPB1* gene, SNP

During the course of evolution, cells have developed complex dynamic mechanisms to respond to many physiological and environmental insults they encounter and subsequently to accumulate heat shock proteins (HSPs) to become more tolerant, a phenomenon termed as thermotolerance (Parsell *et al*. 1993). Isoforms of HSPs are categorized into 5 major families on the basis of their size, structure and function, viz. the HSP110, HSP90, HSP70, HSP60 and small HSP family (Lindquist and Craig 1988, Kregel 2002). Intracellular *HSP27* (heat shock 27kDa protein), a member of small HSP family (sHSP), is ubiquitous stress protein with various functions such as ATP-independent chaperone activity in response to environmental stress, control of apoptosis, the efficient inhibition of insulin aggregation, and microfilament stabilization (Landry 1989, Garrido 2001, Kregel 2002, Concannon *et al*. 2003, Bryantsev *et al*. 2007). Heat shock protein beta-1 (*HSPB1*) and heat shock protein beta-2 (*HSPB2*) are 2 genes of *HSP27* gene group in cattle located on BTA25 and BTA15, respectively (Zimin *et al*. 2009). Change in climate as a stressor could be major threat to the future of sustainability of livestock production systems. Deleterious effects of heat stress on reproductive performance in dairy cattle are reviewed by Hansen (2009). Therefore, sustainable strategy of assisting managemental modifications with genetic evaluation of dairy animals for economic traits by utilizing variations in candidate genes associated with a stress response could be successful in reducing effects of stress in long term. *HSP* genes are important candidates for this purpose. Arya *et al.* (2016) reported bovine *HSPB1* gene polymorphism in Murrah buffalo. However, there is no report on bovine *HSPB1* genes single nucleotide polymorphisms (SNPs) detection in Karan-Fries and Sahiwal cattle which are the important cattle breeds in India. Therefore, the present study was planned to screen SNPs in bovine *HSPB1* gene, and to find its effect on age at calving of two dairy cattle breeds in India.

MATERIALS AND METHODS

All experimental procedures received prior approval by the Institutional Animal Ethics Committee of Indian Council of Agricultural Research-National Dairy Research Institute

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(ICAR-NDRI), Karnal, India. A total of 180 clinically healthy cows calved normally in first three calving were randomly selected for blood sample collection, and age at each calving for each of those cows was calculated in months using records of individual cow available at ICAR-NDRI.

Genomic DNA was extracted for all cows from whole blood using phenol-chloroform extraction method described by Sambrook *et al.* (2001) with minor modifications. Based on publically available bovine *HSPB1* gene sequence (Ensembl Number: ENSBTAT00000044397), one primer set (Hsp27_-75Forward; 5'-GCCCCGCGCCCTGGTATG-3' and Hsp27_461Reverse; 5'-TCAAGACCAAGGACGG CG-3') was designed and commercially synthesized at Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, and used for DNA amplification of the 536 bp target region via polymerase chain reaction (PCR) using thermal cycler (BioRad T100). Targeted region of bovine *HSPB1* gene covered upstream sequence (75 bp), 5' UTR (124 bp) and partial first coding region (337 bp). Each PCR began with initial denaturation at 94°C for 3 min followed by 34 cycles of 94°C for 30 sec, 63°C for 30 sec and 72°C for 30 sec. A final elongation step consisted of 10 min at 72°C. Amplicons were analyzed for fragment integrity by electrophoresis in agarose gels (2% w/v in 0.5× Tris Boric acid EDTA buffer) stained with ethidium bromide. Further, amplicons were purified and sequenced at First BASE Laboratories, Singapore. To detect SNPs, reference sequence was compared with sequencing results of each cow using the web-based software package CLUSTAL MUSCLE.

All population genetics analyses were performed using the POPGENE software package (Yeh *et al*. 1999). Association of individual SNP genotypes of bovine *HSPB1* gene with age at calving was analyzed separately in both breeds via regression using a repeated gene substitution MIXED model and least-squares means (SAS Inst. Inc., Cary, NC, USA). Following statistical model was used

$$Y_{ij} = \mu + C_i + \Sigma\, b_k(X_{ij}) + e_{ij}$$

where, Y_{ij}, phenotypic value of age at calving; μ, overall mean; C_i, fixed effect of i^{th} calving; b_k, regression coefficient on number of copies of significant alleles of bovine *HSPB1*gene; X_{ij}, copies of alleles of significant SNPs within bovine *HSPB1* gene; and e_{ijk}, random error.

RESULTS AND DISCUSSION

Distribution of SNPs within targeted region of bovine *HSPB1* gene revealed a transition of thymine to cytosine at SNP rs208395876 within 5' UTR (Figs 1, 2) and a silent transversion of guanine to thymine at SNP rs723061520

Table 1. Genotype and allele frequency distribution at bovine *HSPB1* gene in Karan-Fries and Sahiwal cattle

Region	dbSNP database reference number	Genotype	Genotype frequencies		Alleles	Allele frequencies	
			Karan-Fries	Sahiwal		Karan-Fries	Sahiwal
5' UTR	rs208395876	TT	0.444*	0.000	T	0.633	0.000
		TC	0.378*	0.000	C	0.367	1.000
		CC	0.178*	1.000			
Exon 1	rs723061520	GG	0.989*	0.678*	G	0.994	0.828
		GT	0.011*	0.300*	T	0.006	0.172
		TT	0.000	0.022*			

*Nonsignificant for HWE.

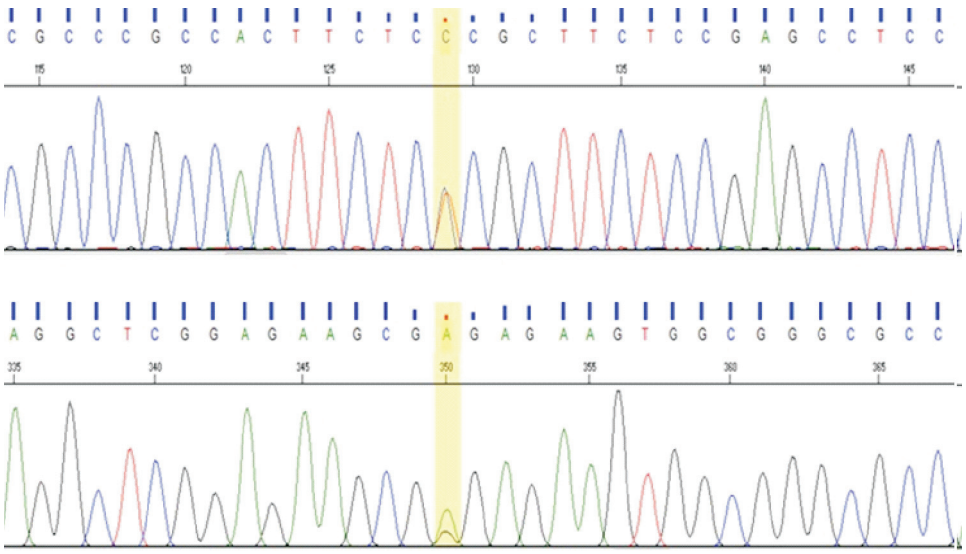

Fig. 1. Genotype TC at SNP rs208395876 in bovine *HSPB1* gene.

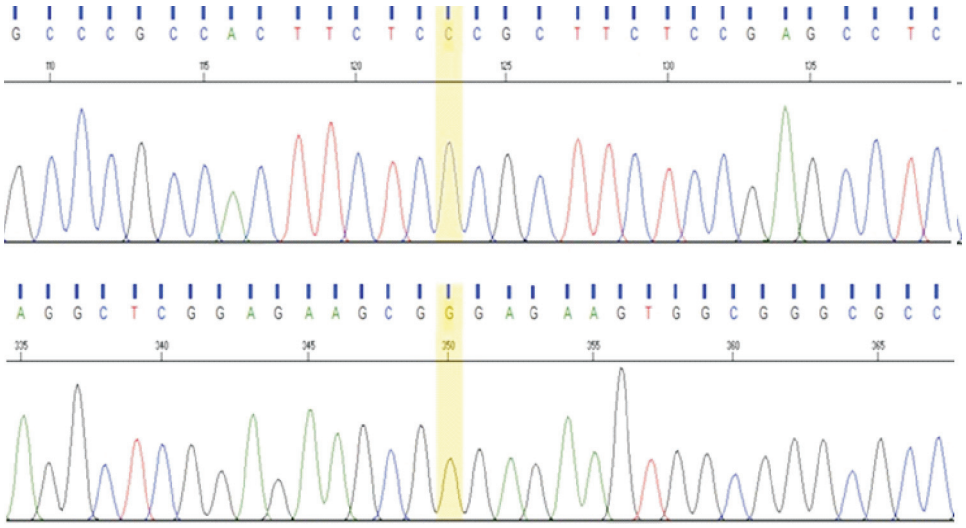

Fig. 2. Genotype CC at SNP rs208395876 in bovine *HSPB1* gene.

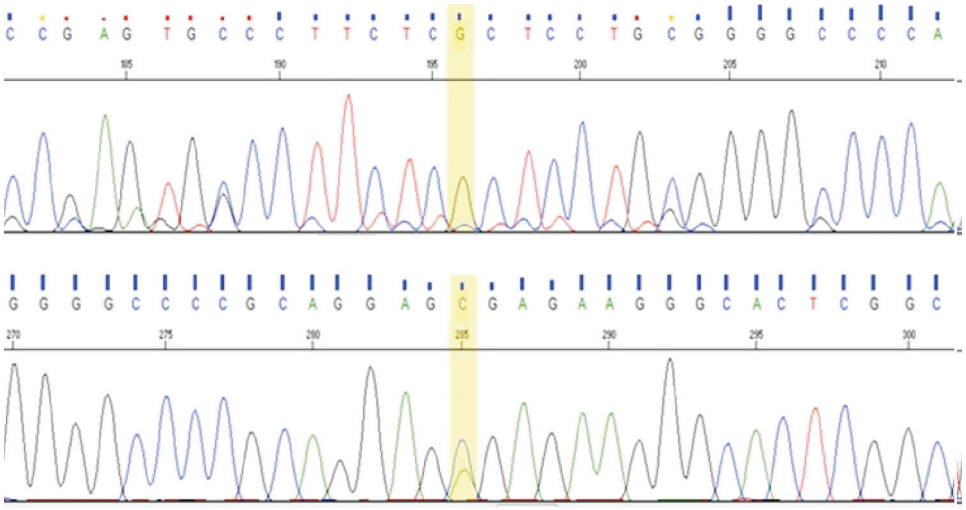

Fig. 3. Genotype GT at SNP rs723061520 in bovine *HSPB1* gene.

within coding sequence (Figs 3, 4) in both Karan-Fries and Sahiwal breeds. Sequences have been submitted and published at Genbank with accession numbers KX377122.1 and KX377121.1, respectively, for Karan-Fries and Sahiwal cattle breeds.

Genotype and allele frequencies and their accordance with or departure from Hardy-Weinberg proportions (HWE) are shown in Table 1. Test for HWE revealed both loci (SNP rs208395876 and SNP rs723061520) in Karan-Fries cows, and one locus (SNP rs723061520) in Sahiwal cows were in equilibrium. In Karan-Fries, breed at SNP locus rs723061520, minor allele was rare and it was present in only heterozygote form. Whereas, minor allele "C" at SNP locus rs208395876 was fixed in Sahiwal breed (Table 1).The difference between observed and expected heterozygosity (H_o<H_E) and positive value of F_{IS} revealed heterozygote deficiency for locus rs208395876 in Karan-Fries breed. Whereas, at locus rs723061520, the observed heterozygosity was greater than expected along with negative value of F_{IS} indicating heterozygote excess in Sahiwal breed (Table 2). Summary of F-Statistics with positive values of F_{IT} (between population and between 2 loci of the 2 breeds) and F_{IS} showed the deficiency of heterozygotes in populations and that mates are more related in comparison with average relationship of the population. The overall observed heterozygosity in both breeds and the F-Statistics values indicated that there is lesser genetic diversity in studied genomic region of bovine *HSPB1* gene in *Bos indicus* compared to crossbred cattle (Table 2). Therefore, it agrees with Hansen (2004) that during evolutionary divergence from *Bos taurus*, *Bos indicus* cattle acquired genes responsible for genetic adaptations to acclimatize harsh tropical environment. *Bos indicus* cows have delayed age at calving (Cunningham and Syrstad 1987) and are generally better adapted to heat stress than *Bos taurus* (Beatty *et al*. 2006). Therefore, question arises, is genetic adaptations of *Bos indicus* cattle to acclimatize harsh tropical environment at the expense of reproduction performance at normal conditions?

Age at calving is one of the important reproductive traits

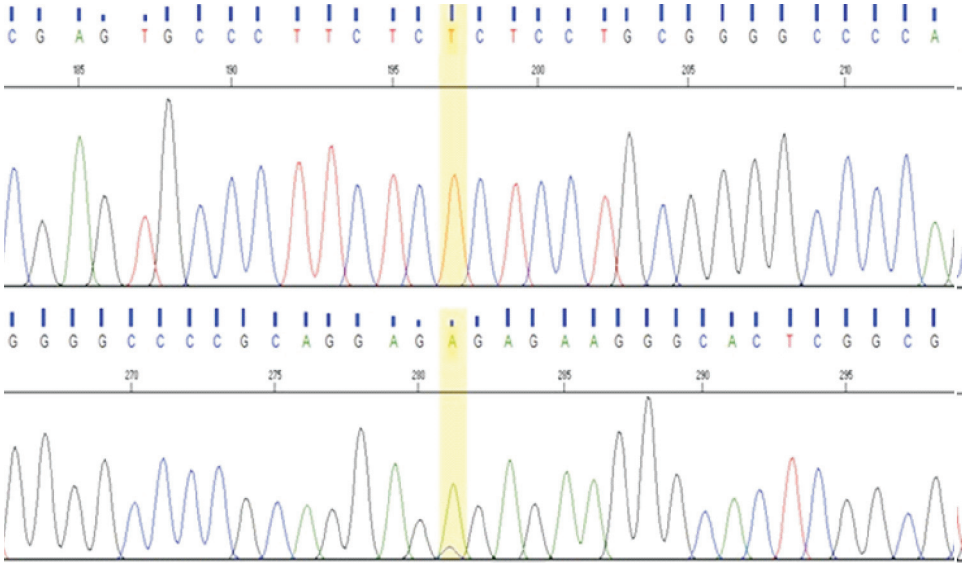

Fig. 4. Genotype TT at SNP rs723061520 in bovine *HSPB1* gene.

in cattle, which gives an indication about the ability to conceive and give birth to a calf, and aids in the earlier evaluation of animals which ultimately reduce the generation interval and helps in faster genetic improvement. Therefore, association analysis of polymorphisms in bovine *HSPB1* gene with age at calving trait was performed. Stepwise regression analysis showed that SNP rs208395876 was significantly associated with age at calving in Karan-Fries cows (Table 3). Further, the gene substitution model confirmed it that there was a significant effect of calving number and SNP rs208395876 on age at calving in Karan-Fries cows (Table 4). However, SNP rs723061520 was not associated with age at calving in both Karan-Fries and Sahiwal cattle. Interestingly, allele C at SNP rs208395876 was present in 36% of Karan-Fries and 100% Sahiwal cows with delayed age at calving compared to remaining cows. Therefore, fixing of the allele C at SNP rs208395876 and delayed age at calving in Sahiwal cows might be related. This effect of bovine *HSPB1* gene with age at calving is further evident with the link between sHSPs and insulin, and between insulin and female reproductive performance. sHSP such as HSP27 have molecular chaperone activity on insulin (Kato *et al*. 1994). Also sHSPs and insulin are linked to ageing process but they act in opposite direction (Hsu *et al*. 2003, Petersen *et al*. 2003). Similarly, Dunaif (1997), Legro (2001), Diamanti and Kandarakis (2003) and

Table 2. Within and between genetic diversity measures at bovine *HSPB1* gene in Karan-Fries and Sahiwal cattle

Locus	Karan-Fries			Sahiwal			F-Statistics			
	H_O	H_E	F_{IS}	H_O	H_E	F_{IS}	F_{IS}	F_{IT}	F_{ST}	Nm
rs208395876	0.3778	0.4670	0.1866	0.0000	0.0000	-	0.1866	0.5636	0.4634	0.2896
rs723061520	0.0111	0.0111	-0.0056	0.3000	0.2867	-0.0522	-0.0504	0.0396	0.0857	2.6656
Mean	0.1944	0.2391	0.0905	0.1500	0.1434	-0.0261	0.0943	0.4209	0.3606	0.4434

H_O, Observed heterozygosity; H_E, expected heterozygosity; F_{IS}, Wright's (1978) fixation index; Nm, gene flow.

Table 3. Effects of SNPs at bovine *HSPB1* gene on age at calving using the stepwise regression analysis

Trait	Breed	Model p-Value	Intercept[a]	SNPs[b,c]			
				rs208395876		rs723061520	
				Estimate (SE)	p-Value	Estimate (SE)	p-Value
Age at calving	Karan-Fries	<.0001	47.31	4.87(1.21)	<.0001	ns	ns
	Sahiwal	ns	-	nd	nd	ns	ns

SE, standard error; ns, nonsignificant; nd, not included in model due to fixing of minor allele; [a]Intercept for both SNPs. The estimates account for the presence of one copy of the C allele. Thus, the estimate has to be doubled for CC genotype. [b]To determine which combination of the genotype, SNPs were independently associated with age at calving trait, a step-wise regression analysis was conducted (SLE = 0.15 and SLS = 0.15) with each SNP. [c]Regression coefficients were estimated by considering both SNPs in model.

Table 4. Least squares means for age at calving, regression coefficient for SNP genotypes and level of significance in Karan-Fries cattle[a]

Trait	Breed	Calving[b]			SNPs[c,d]		ANOVA significance level[p (F)]			
		First	Second	Third	rs208395876	rs723061520	Calving		SNP	
							Type I	Type III	rs208395876	rs723061520
Age at calving	Karan-Fries	34.69^{A} (0.44)	50.87^{B} (0.69)	67.09^{C} (0.89)	4.44 (1.20)*	nd	<.0001	<.0001	<.0001	nd
	Sahiwal	38.77^{A} (0.79)	53.50^{B} (0.79)	68.64^{C} (0.79)	nd	nd	nd	nd	nd	nd

Means within a factor with different superscript capital letters differ significantly (P<0.05); nd, not included in model due to nonsignificance in step-wise regression analyses; figures in parenthesis are SE; [a]statistical analysis carried out using calving as fixed class variable and only fixed significant SNP derived from stepwise regression analysis; [b]least squares means with SE were estimated using statistical models by considering only significant factors; [c]regression coefficients with SE were estimated by considering all variables in the model; *Significant effect of SNP on Type III analysis; [d]variable calving placed always in the first position in mode.

Nandi *et al.* (2010) reported relationship between altered insulin function and female fertility. Therefore, additional research will be required to determine physiological mechanisms involved in interrelation between sHSPs, insulin, ageing and cow fertility.

In conclusion, our results indicated that the studied genomic region of bovine *HSPB1* gene is polymorphic. In addition, these polymorphisms were informative with regard to age at calving of crossbred cows. Therefore, this gene is an important candidate for cow fertility. Additional research will be required to establish the physiological mechanisms by which these polymorphisms alter calving age in cattle, to evaluate effect of adjoining regions of these polymorphisms and to evaluate genotype-environment interactions.

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