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ORIGINAL RESEARCH

Polymorphisms in the bovine *HSP90AB1* gene are associated with heat tolerance in Thai indigenous cattle

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Abstract Heat shock proteins act as molecular chaperones that have preferentially been transcribed in response to severe perturbations of the cellular homeostasis such as heat stress. Here the traits respiration rate (RR), rectal temperature (RT), pack cell volume (PCV) and the individual heat tolerance coefficient (HTC) were recorded as physiological responses on heat stress (environmental temperatures) in *Bos taurus* (crossbred Holstein Friesian; HF) and *B. indicus* (Thai native cattle: White Lamphun; WL and Mountain cattle; MT) animals (n=47) in Thailand. Polymorphisms of the heat shock protein 90-kDa beta gene (*HSP90AB1*) were evaluated by comparative sequencing. Nine single nucleotide polymorphisms (SNP) were identified, i.e. three in exons 10 and 11, five in introns 8, 9, 10 and 11, and one in the 3′UTR. The exon 11 SNP g.5082C>T led to a missense mutation (alanine to valine). During the period of extreme heat (in the afternoon) RR and RT were elevated in each of the three breeds, whereas the PCV decreased. Mountain cattle and White Lamphun heifers recorded significantly better physiologic parameters ($p<0.05$) in all traits considered, including or particularly HTC than Holstein Friesian heifers. The association analysis revealed that the T allele at SNP g.4338T>C within intron 3 improved the heat tolerance ($p<0.05$). Allele T was exclusively found in White Lamphun animals and to 84% in Mountain cattle. Holstein Friesian heifers revealed an allele frequency of only 18%. Polymorphisms within *HSP90AB1* were not causative for the physiological responses; however, we propose that they should at least be used as genetic markers to select appropriate breeds for hot climates.

Keywords Heat stress · *HSP90AB1* · Polymorphisms · Indigenous cattle · Thailand

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Introduction

Global warming and the proposed climate change are likely to become the major threats to the sustainability of livestock production systems in the future. Simulations of different climate scenarios suggest regional rises in temperature. In addition, the intensity and duration of heat waves will dramatically increase (Gaughan et al. 2010). The changes will cause pertinent heat stress to livestock visible as predicted by, e.g. a reduced feed consumption rate (Bernabucci et al. 1999), a decreased milk production (Sharma et al. 1988) and a lower reproductive success rate (Cavestany et al. 1985).

The physiological mechanisms of heat stress regulation are known to be identical in *Bos taurus* and *B. indicus* cattle. Temperatures above the comfort zone, a high relative humidity and an intensive solar radiation alone or together with a low wind speed are the primary factors that increase an individual's heat load and finally cause (heat) stress (Mader et al. 2006). *B. indicus* is, however, generally better adapted to heat stress (Beatty et al. 2006): several studies report that the detrimental effects of heat stress on production traits are of a lesser extent (Gaughan et al. 1999; Gaughan et al. 2010). Mammals respond to heat stress with an evolutionary old and conserved adaptive cellular system. It is characterized by the transcriptional activation and accumulation of a set of proteins known as heat shock proteins (HSP). Isoforms of these proteins are categorized into families with respect to their molecular weight, i.e. HSP27, HSP60, HSP70, HSP90 and HSP110/104 (Kregel 2002). Proposed functions of HSPs are microfilament stabilization (HSP27), refolding of proteins and prevention of aggregation of denatured proteins (HSP60), regulation of steroid hormone receptors and protein translocation (HSP90) and protein folding (HSP110/104). HSP70 proteins are molecular chaperons. HSP27 and HSP70 proteins have antiapoptotic and HSP60 proteins proapoptotic effects (Kregel 2002).

Ninety-kilodalton heat shock proteins (Hsp90) act as important molecular chaperones that are constitutively expressed as a consequence of heat or stress induction (Chen et al. 2006). Two major cytoplasmatic Hsp90 isoforms are constituted by gene duplication: the inducible Hsp90α and the constitutive Hsp90β form. The contribution of Hsp90 isoforms to various cellular processes including signal transduction, protein folding, protein degradation, cell survival and morphological evolution has extensively been studied (Csermely et al. 1998).

The 'trait' heat tolerance is a quantitative trait (Gaughan et al. 2010; Li et al. 2011; Liu et al. 2011). Several studies aimed to find the link between phenotypes and genotypes. A quantitative trait locus (QTL) study in *Drosophila melanogaster* (Morgan and Mackay 2006) mapped heat stress resistance to a genomic region on chromosome 3 containing amongst other genes the positional candidate gene *HSP83*, which is the ortholog to the mammalian *HSP90* gene family (Marcos-Carcavilla et al. 2010). In sheep, polymorphisms within another Hsp90 gene, the *HSPAA1*, were investigated. A single nucleotide polymorphism (SNP) located at position −660 in the 5′flanking region was associated with different thermal conditions (Marcos-Carcavilla et al. 2010). An SNP at nucleotide position 2789 within *ATP1A1* messenger RNA is known to be associated with heat tolerance traits in dairy cows (Liu et al. 2010; Liu et al. 2011). Effects of the SNP g.1524G>A, g.3494T>C and g.6601G>A within *HSP70A1A* affect thermo-tolerance in Chinese Holstein cattle (Li et al. 2011). However, there have been no reports of genetic variations in bovine HSP90 genes and heat tolerance.

The objective of this study was to record physiological parameters along with heat stress, to search for sequence variants in *HSP90AB1* and to describe putative associations between them in three cattle breeds used in Northern Thailand.

Materials and methods

Experimental animals

Forty-seven clinically healthy not lactating females between 12 and 18 months were randomly selected and kept at the experimental farm of the Chiang Mai University in Thailand. The animals had different genetic backgrounds and belonged to the indigenous *B. indicus* breeds White Lamphun (WL; $n=17$) and Mountain cattle (MT; $n=16$). Fourteen crossbreds (Holstein Friesian (HF)×Thai native breed) were further used. As the proportion of Holstein Friesian blood was individually between 82.8% and 98.4%, these animals are further regarded as Holstein Friesian heifers. The cattle were kept in groups according to the animal welfare rules at the experimental farm under natural conditions. They were fed ad libitum on seasonal grass, rice straw and fresh water.

Physiological parameters

Respiratory rate (diaphragm movements per minute) (RR) and rectal temperature (RT) (°C) were measured in the morning (8.00 a.m.) and in the afternoon (2.00 p.m.). In addition, blood samples were collected according to the recommendation of the manufacturer in microhematocrit capillaries to measure the pack cell volume (PCV) using a hematocrit centrifuge at 10000 rpm for 5 min (HAEMATOKRIT-210; Hettich, Germany). The measurements and the sample collection were performed 2 weeks per month for four consecutive months (September to December, i.e. from the end of the rainy season until the middle of the winter season) to achieve eight observations per animal. The outdoor temperature and the relative humidity (RH) (%) were recorded daily during the experiment.

Earlier heat tolerance experiments led to the development of a formula (Rhoad 1944) to calculate an individual's heat tolerance coefficient (HTC). This formula, also known as the Iberia heat tolerance test for cattle, is as follows:

$$\mathrm{HTC} = 100 - 10(\mathrm{ART} - 38.3),$$

where HTC is the heat tolerance coefficient, ART is the average rectal temperature, 38.3 is the physiological bovine body temperature, 10 is a correction factor to convert deviations in body temperature to a unit basis and 100 is the perfect efficiency in maintaining temperature at 38.3°C. The index of HTC was calculated for each cow to assess its heat adaptability.

Molecular genetics

Genomic DNA was extracted for all experimental animals from whole blood (9-ml vials containing EDTA) and/or from small pieces of tissue taken from the ears with a modified salting out method according to Sambrook et al. (1989) and Miller et al. (1988). Twelve DNA samples (four HF, four WL and four MT) of Thai heifers and 12 samples (four German Holstein Friesians, four Holstein Reds and four Charolais) of the DNA repository at the Institute of Veterinary Medicine in Göttingen (Germany) were randomly chosen to screen for SNP by comparative sequencing.

Six primer combinations were created based on the publicly available bovine *HSP90AB1* gene sequence (Acc. No. NW001494158). The PCR products cover exons 2 to 12 (Table 1). PCR was carried out using 50 to 100 ng of genomic DNA, 0.2 mM dNTPs, 40 pM of each primer and 2.5 U of Taq DNA polymerase in 1× PCR buffer in a final volume of 25 µl. The PCR profile used was: 35 cycles at 94°C for 30 s, a primer specific annealing temperature (see Table 1) for 30 s and an extension period of 30 s at 72°C with an initial denaturation for 2 min at 94°C and a final extension at 72°C for 5 min. PCR reactions were performed using the Biometra T-Gradient thermocycler (Biometra, Germany). To check fragment integrity PCR products were separated on 1% agarose gels. PCR products were then purified with the QIAquick PCR Purification Kit (Qiagen, Germany). The purified PCR products were directly sequenced using the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit® (Applied Biosystems, Germany) on an automated DNA sequencer (ABI-PRISM 3100® capillary analyzer; Applied Biosystems, Germany). The sequenced data were analyzed and manually checked using the software suite DNASTAR LasergeneTM 6® (DNASTAR, Inc., Germany).

Statistical analysis

Gene diversity, allele and genotype frequencies and their accordance with or deviation from the Hardy–Weinberg law were determined by POPGENE 1.31 (Yeh et al. 1999) and GenAlEx 6.3 (Peakall and Smouse 2006). For each trait, association analyses via regression on individual SNP genotypes, a repeated gene substitution MIXED model and least square means (SAS Inst., Inc., Cary, NC, USA) were performed. In the first step any possible association between an SNP within the *HSP90AB1* gene and a trait was analyzed using stepwise regression analysis. In the second step a gene substitution model was used to analyze breed specific effects and those of significant SNPs driven from the stepwise regression. The following model was applied:

$$Y_{ij} = \mu + B_i + \sum_k b_k\left(X_{ij}\right) + e_{ijk},$$

where Y_{ij} is the phenotypic value of heat associated traits, μ is the overall mean, B_i is the fixed effect of ith breed, b_k is the regression coefficient on the number of copies of significant alleles of *HSP90AB1* gene and k is the number of significant SNPs of the *HSP90AB1* gene. X_{ij} presents the copies of alleles of significant SNPs within *HSP90AB1*, and e_{ij} is the random error. The sire effect was not included in the statistical model. Significance level of differences among genotype groups was determined at $p<0.05$.

Table 1 Primers for comparative sequencing of *HSP90AB1*

Primer	Primer sequences (5′→3′)	Position[a]	Size (bp)	Tm (°C)
Ex2Af	CCTGGATTGGAATGCCTAAC	1160	734	61.6
Ex3Br	TCAGGCTCTCATAGCGAATC	1894		
Ex3Af	AGGGAGTAATCAGAATAAG	1777	934	58.7
Ex5Ar	AGATGACAGTTTCAGAGTG	2711		
Ex6Af	TCACCCAGGAGGAATATGGAG	2981	692	61.6
Ex8Br	AGAAGGACCGATTTTCTCACC	3673		
Ex8Af	TTAAGGATCCTCTGCAGCAC	3638	710	61.6
Ex10Br	GCAACCTGCTCTTTGCTCTC	4348		
Ex9Af	TCTATTACATCACTGGTGCG	4207	654	61.6
Ex10Cr	TGTTGGAGATCGTCACCTG	4861		
Ex10Af	AGGTGGAGAAGGTAAGCCATT	4604	1040	62.9
Ex12Br	GTGTAAAAAACCAGCATCTTC	5664		

Tm temperature

[a]Numbers refer to GenBank Acc. No. NW001494158

Results and discussion

Polymorphism screen and population genetics parameters

Nine novel polymorphisms − SNP01 to SNP09 (three in exons, five in introns and one in the 3′UTR) − covering 5,664 bp of the bovine *HSP90AB1* were detected by comparative sequencing of 24 animals representing the six breeds. SNP07 led to a missense mutation (alanine to valine); the further SNPs proved to be silent. Allele and genotype frequencies are displayed in Table 2. Fixed allele frequencies were predominantly found in the HF heifers, and the most balanced distribution of alleles over all data displayed the Mountain cattle. A close to 1:1 ratio of alleles was only found for four SNPs (SNP04, SNP06 and SNP09 in Mountain cattle, and SNP08 in White Lamphun). The calculated genetic heterozygosity based on allele frequencies was low in Holstein Friesian (0.071) but high in Thai native cattle (0.326 for Mountain cattle and one of 0.307 for White Lamphun). The data support therefore a higher genetic diversity of Thai native cattle as proposed before by the Department of Livestock Development in Thailand (Boonyanuwat et al. 2005). The authors calculated mean heterozygosities between 0.415 and 0.565 after analysis of 30 STS loci in four Thai indigenous cattle (including White Lamphun).

Physiological parameters and their associations with *HSP90AB1* sequence variants

Associations between sequence variants within *HSP90AB1* and physiological parameters were analyzed. Earlier, others have considered RR, PCV, RT and HTC as parameters to

Table 2 Genotype and allele frequencies per breed at *HSP90AB1*

Regions[a]	SNPs	Genotypes	Genotype frequencies (n)				Alleles	Allele frequencies			
			HF	MT	WL	Pooled[b]		HF	MT	WL	Pooled[b]
Intron 8	1) g.4029G>C (SNP01)	CC	0	0	0	0	C	0	0	0.235	0.085
		CG	0	0	0.471 (8)	0.170 (8)	G	1.000	1.000	0.765	0.915
		GG	1.000 (14)	1.000 (16)	0.529 (9)	0.830 (39)					
	2) g.4061G>A (SNP02)	AA	0	0.125 (2)	0.059 (1)	0.064 (3)	A	0	0.313	0.324	0.224
		AG	0	0.375 (6)	0.529 (9)	0.319 (15)	G	1.000	0.688	0.676	0.776
		GG	1.000 (14)	0.500 (8)	0.412 (7)	0.617 (29)					
Intron 9	3) g.4338T>C (SNP03)	CC	0.643 (9)	0	0	0.191 (9)	C	0.821	0.156	0	0.298
		CT	0.357 (5)	0.313 (5)	0	0.213 (10)	T	0.179	0.844	1.000	0.702
		TT	0	0.688 (11)	1.000 (17)	0.596 (28)					
Exon 10	4) g.4374T>G (SNP04)	GG	0	0.313 (5)	0.941 (16)	0.447 (21)	G	0.036	0.594	0.971	0.564
		GT	0.071 (1)	0.563 (9)	0.059 (1)	0.234 (11)	T	0.964	0.406	0.029	0.436
		TT	0.929 (13)	0.125 (2)	0	0.340 (15)					
Intron 10	5) g.4730A>C (SNP05)	AA	1.000 (14)	1.000 (16)	0.588 (10)	0.851 (40)	A	1.000	1.000	0.794	0.925
		AC	0	0	0.412 (7)	0.149 (7)	C	0	0	0.206	0.075
		CC	0	0	0	0					
Exon 11	6) g.5007T>C (SNP06)	CC	0	0.313 (5)	0.941 (16)	0.447 (21)	C	0.036	0.563	0.971	0.553
		CT	0.071 (1)	0.500 (8)	0.059 (1)	0.213 (10)	T	0.964	0.438	0.029	0.447
		TT	0.929 (13)	0.188 (3)	0	0.340 (16)					
	7) g.5082C>T (SNP07)	CC	1.000 (14)	0.563 (9)	0.882 (15)	0.809 (38)	C	1.000	0.719	0.941	0.883
		CT	0	0.313 (5)	0.118 (2)	0.149 (7)	T	0	0.281	0.059	0.117
		TT	0	0.125 (2)	0	0.043 (2)					
Intron 11	8) g.5248C>T (SNP08)	CC	0.929 (13)	0.563 (9)	0.353 (6)	0.596 (28)	C	0.964	0.781	0.588	0.766
		CT	0.071 (1)	0.438 (7)	0.471 (8)	0.340 (16)	T	0.036	0.219	0.412	0.234
		TT	0	0	0.176 (3)	0.064 (3)					
3′UTR	9) g.5435T>C (SNP09)	CC	0	0.313 (5)	0.941 (16)	0.447 (21)	C	0.036	0.531	0.971	0.543
		CT	0.071 (1)	0.438 (7)	0.059 (1)	0.191 (9)	T	0.964	0.469	0.029	0.457
		TT	0.929 (13)	0.250 (4)	0	0.362 (17)					

[a] Numbers refer to GenBank Acc. No. NW001494158

[b] Values of the total number of White Lamphun heifers, Mountain cattle heifers and Holstein Friesian heifers that were investigated

evaluate the heat stress/tolerance of cattle (Beatty et al. 2006; Liu et al. 2010; Liu et al. 2011). We elaborated the parameters further to define the traits AM-RR (respiratory rate in the morning), PM-RR (respiratory rate in the afternoon), AM-PCV (blood pack cell volume in the morning), PM-PCV (blood pack cell volume in the afternoon), AM-RT (rectal temperature in the morning) and PM-RT (rectal temperature in the afternoon). In addition, all recorded observations to calculate average values for RR, PCV and RT (ARR, APCV and ART) were used. During the experimental time an averaged hot and humid climate of 22°C and 94% RH in the morning increased to 34°C and 68% RH in the afternoon. During the extreme heat in the afternoon, RR and RT traits were elevated in animals of all of the breeds, whereas all traits corresponding to the PCV decreased compared to the morning values. Mountain cattle and White Lamphun heifers recorded significantly better physiologic parameters ($p<0.05$) in all traits considered, including or particularly HTC than Holstein Friesian heifers (98.38 and 96.85 compared to 95.28) (Table 4). Table 3 summarizes the effects of the SNP on the physiological parameters using the stepwise regression analysis. To determine which of the nine SNPs were associated with the traits, a forward stepwise regression analysis was conducted ($\alpha=0.05$ for inclusion and 0.05 exclusion). For five (SNP03, SNP06, SNP07, SNP08 and SNP09) of the nine SNPs, an effect on at least one of the ten traits was computed. SNP03 was the most frequently associated DNA variation (effects on eight of the ten traits) with as well the highest significance thresholds. Effects of miscellaneous SNP on a trait were evident, except for the RR traits.

HSP90AB1 SNP and RR traits

An increased RR is an important thermoregulatory response to heat stress. It aids in heat dissipation via evaporative cooling (Hammond et al. 1996; Beatty et al. 2006). Thus, a low RR may indicate an improved thermotolerance. Using the stepwise regression model, we report associations between SNP03 and SNP06 on RR traits approved by $p<0.05$ or better. The possession of one T allele at SNP06 increased the AM-RR by 3.24 times/min ($p<0.0001$), whereas one copy of the T allele at SNP03 lowered the PM-RR (−2.68 times/min) as well as the ARR (−3.10 times/min). The allele frequency distributions indicate a high frequency of the detrimental SNP06 T allele (0.96) in Holstein Friesian heifers but elevated frequencies of the supportive T allele at SNP03 in the indigenous Thai cattle. Contrary to that, the gene

Table 3 Effects of SNPs on heat stress response traits using the stepwise regression analysis

Traits	Intercept[a]	SNPs[b,c]									
		SNP03		SNP06		SNP07		SNP08		SNP09	
		Estimate (SE)	*p*-Value	Estimate (SE)	*p*-Value	Estimate (SE)	*p*-Value	Estimate (SE)	*p*-Value	Estimate (SE)	*p*-Value
AM-RR (times/min)	21.45	ns	ns	3.24 (0.67)	0.0001	ns	ns	ns	ns	ns	ns
PM-RR (times/min)	41.86	−2.68 (1.23)	0.035	ns	ns	ns	ns	ns	ns	ns	ns
ARR (times/min)	35.59	−3.10 (0.83)	0.0006	ns	ns	ns	ns	ns	ns	ns	ns
AM-PCV (%)	26.96	4.59 (0.85)	0.0001	ns	ns	3.12 (1.31)	0.022	ns	ns	ns	ns
PM-PCV (%)	24.70	3.10 (0.74)	0.0001	ns	ns	2.62 (1.14)	0.027	ns	ns	ns	ns
APCV (%)	26.76	3.86 (0.76)	0.0001	ns	ns	2.87 (1.18)	0.019	ns	ns	ns	ns
AM-RT (°C)	37.96	ns	ns	1.10 (0.28)	0.0004	ns	ns	ns	ns	−0.99 (0.28)	0.001
PM-RT (°C)	39.60	−0.28 (0.09)	0.006	ns	ns	ns	ns	0.23 (0.09)	0.012	−0.18 (0.09)	0.043
ART (°C)	38.69	−0.11 (0.04)	0.019	ns	ns	ns	ns	0.16 (0.06)	0.009	ns	ns
HTC	96.10	1.09 (0.44)	0.019	ns	ns	ns	ns	−1.57 (0.57)	0.009	ns	ns

ns not significant

[a] Intercept is for all five SNPs the trait mean of the genotype CC. The estimates account for the presence of one copy of the T allele. Thus, the estimate has to be doubled for TT genotypes

[b] To determine which combination of the nine genotype SNPs were independently associated with different heat tolerant traits, a forward stepwise regression analysis was conducted ($\alpha=0.05$ for inclusion and 0.05 exclusion) with each SNP coded with different genotypes (1st = CC, 2nd = CT and 3rd = TT)

[c] Regression coefficients are estimated by considering all SNPs in model (SNP01 to SNP09)

substitution model (Table 4) suggests that this observation is primarily breed-specific: White Lamphun has lower RR compared to Mountain cattle resp. Holstein Friesian for all three traits that also differ significantly ($p<0.05$) except for AM-RR. For this trait no significant differences between Mountain cattle and White Lamphun do exist. In literature, there is no evidence that under physiological conditions the respiratory capacity to handle heat is superior in zebu cattle. The proportion of evaporation was roughly similar for Brahman, Holstein, Jersey and Brown Swiss. Heat stress, however, enhances the evaporative heat loss via respiration in European breeds (Seif et al. 1979; Gaughan et al. 1999; Gaughan et al. 2010) indicating more sophisticated heat loss mechanisms in less-adapted breeds to higher temperatures (Hansen 2004).

HSP90AB1 SNP and PCV traits

SNP03 resp. SNP07 were significantly ($p<0.001$ resp. $p<0.05$) associated with the PCV traits. In any case, the presence of T allele provided an advantage of 2.62 to 4.50% (Table 3). In turn, the gene substitution model proved that again only the breed attributed significantly to effects on PCV traits. Mountain cattle heifers revealed the highest percentage of recorded PCV, whereas Holstein Friesian heifers showed the lowest and White Lamphun cattle represented medium values. The T allele frequencies are in fact not the highest in the Mountain cattle. Thus, we assume that these two SNPs might act rather as markers than as causative sequence variations for PCV traits. Putative physiological differences of *B. taurus* resp. *B. indicus* cattle to continuous heat and humidity were

Table 4 Least squares means for breeds, regression coefficient for SNP genotypes and level of significance[a]

Traits	Breeds[b]			SNPs[c,d]				
	HF	MT	WL	SNP03	SNP06	SNP07	SNP08	SNP09
AM-RR (times/min)	28.95 (1.01)^{A}	23.85 (0.95)^{B}	21.05 (0.92)^{B}	nd	0.51 (1.20)	nd	nd	nd
PM-RR (times/min)	39.73 (1.76)^{A}	40.20 (1.65)^{A}	34.76 (1.60)^{B}	−4.56 (2.49)	nd	nd	nd	nd
ARR (times/min)	34.34 (1.19)^{A}	32.03 (1.11)^{A}	27.92 (1.08)^{B}	−2.31 (1.71)	nd	nd	nd	nd
AM-PCV (%)	26.53 (0.74)^{A}	39.53 (0.80)^{B}	35.34 (0.78)^{C}	−0.34 (1.27)	nd	0.42 (1.06)	nd	nd
PM-PCV (%)	26.08 (0.71)^{A}	36.44 (0.66)^{B}	31.77 (0.64)^{C}	−0.65 (1.07)	nd	0.03 (0.87)	nd	nd
APCV (%)	26.27 (0.72)^{A}	37.98 (0.67)^{B}	33.55 (0.65)^{C}	−0.48 (1.06)	nd	0.23 (0.88)	nd	nd
AM-RT (°C)	38.20 (0.11)^{A}	37.95 (0.07)^{A}	37.99 (0.10)^{A}	nd	**0.92** (0.30)	nd	nd	**−0.90** (0.28)
PM-RT (°C)	39.07 (0.15)^{A}	39.02 (0.08)^{A}	39.32 (0.11)^{A}	**−0.26** (0.12)	nd	nd	**0.22** (0.08)	−0.08 (0.10)
ART (°C)	38.77 (0.55)^{A}	38.46 (0.05)^{B}	38.61 (0.05)^{AB}	0.01 (0.07)	nd	nd	**0.13** (0.05)	nd
HTC	95.28 (0.54)^{A}	98.38 (0.47)^{B}	96.85 (0.49)^{AB}	−0.07 (0.76)	nd	nd	**−1.33** (0.53)	nd
	ANOVA significance level [P (*F*)]							
	Breeds		SNPs					
	Type I[d]	Type III	SNP03	SNP06	SNP07	SNP08	SNP09	
AM-RR (times/min)	<0.0001	0.041	nd	0.681	nd	nd	nd	
PM-RR (times/min)	0.036	0.077	0.075	nd	nd	nd	nd	
ARR (times/min)	<0.0001	0.123	0.183	nd	nd	nd	nd	
AM-PCV (%)	<0.0001	<0.0001	0.783	nd	0.688	nd	nd	
PM-PCV (%)	<0.0001	<0.0001	0.541	nd	0.973	nd	nd	
APCV (%)	<0.0001	<0.0001	0.648	nd	0.797	nd	nd	
AM-RT (°C)	0.006	0.203	nd	**0.004**	nd	nd	**0.003**	
PM-RT (°C)	0.005	0.089	**0.049**	nd	nd	**0.016**	0.458	
ART (°C)	0.002	0.008	0.915	nd	nd	**0.017**	nd	
HTC	0.002	0.008	0.926	nd	nd	**0.017**	nd	

Means within a factor with different superscript capital letters differ significantly ($p<0.05$)

nd not included in model due to non-significance in stepwise regression analyses

[a] Statistical analysis carried out using breed as fixed class variable and only fixed significant SNPs derived from stepwise regression analysis

[b] Ls-means with standard error (SE) are estimated using statistical models by considering only significant factors

[c] Regression coefficients with standard error (SE) are estimated by considering all variables in the model; *bold* is a significant effect of SNPs on Type III analysis

[d] The variable breed placed always in the first position in model

investigated previously by Beatty et al. (2006). The authors propose that the increased water consumption under higher temperatures will lead to an increased total blood volume and a decrease in PCV. We did not measure the total blood volume, the water intake − and also not the water output as urine − to assure this observation but conclude that Mountain cattle heifers consume less water to keep the homeostasis compared to the other two breeds.

HSP90AB1 SNP and RT traits

Most associations between SNPs within *HSP90AB1* and traits were recorded for rectal temperature. These effects were highly significant but at the same time also inconsistent as well. In total, nine putative SNP effects existed. The stepwise regression analysis revealed effects of the T allele on the trait AM-RT: SNP06 accounts for a temperature raise of 1.10°C, and a decrease of 0.99°C is at the same time caused by a T allele at SNP09. The T and C allele frequency at the loci SNP06 and SNP09 is identical in Holstein Friesian and White Lamphun but not the ones for SNPs 07 and 08 that are physically lying in between. As the SNP positions are only 428 bp away from each other, recombination events in this gene area are possible. The estimated regression coefficient decreased for the traits PM-RT resp. ART in the presence of a T allele at SNP03 by 0.28°C (p=0.006) resp. by 0.11°C (p=0.019). A T allele at SNP07 was associated with a temperature increase of 0.23°C (p=0.012) resp. 0.16°C (p=0.009). Preferred HTCs are associated with the T allele at SNP03, and a detrimental effect on this trait comes from variant T at SNP08 (Table 3). The gene substitution model (Table 4) finally proved that only RT traits depend on the breed and the investigated SNP.

Do *HSP90AB1* SNPs contribute to heat stress/heat tolerance in Thai cattle breeds?

Thailand is located on the Indi–China peninsula. The climate is monsoonal tropical that remains hot and humid throughout the year. The average temperature is about 29°C, ranking in Bangkok (capital city) from 35°C in April to 17°C in December (MFA 2011). White Lamphun and the Mountain cattle are the most prominent native cattle breeds in Northern Thailand. They are rather fertile animals, tolerant towards a poor food quality and also towards internal and external parasites (Rattanaronchart 1998). The breeds are well adapted to the environment, but there were very few studies to prove this both with phenotypic and genetic data.

Here in no case, the values of the investigated physical parameters were pathological, but the data clearly underline a superior performance of Mountain cattle and White Lamphun compared to the *B. taurus* individuals. There are several physiological mechanisms to cope with heat stress (i.e. sweating, high respiratory rate, rising rectal temperature above critical thresholds, increased water consumption, reduced metabolic rate and a decreased dry matter intake) that at the same time reveal a negative impact on the production and reproduction performance of the cattle (West 2003; Hansen 2004; Beatty et al. 2006). It is well described that all of these physiological responses are substantially enhanced in *B. taurus* compared to *B. indicus* (Hammond et al. 1996; Collier et al. 2008). In addition, there is also ample evidence that the basal metabolic rate of *B. indicus* is generally lower compared to *B. taurus* (Gaughan et al. 1999; Hansen 2004; Gaughan et al. 2010). Clearly, low metabolic rates are consequences of reduced or low performance traits such as growth rate and milk yield. Thus, low producing cattle (livestock) reveal an increased heat tolerance (Reid et al. 1991). However, to finally cope well with heat stress further factors including housing, nutrition, health status, age and body condition have to be considered (Gaughan et al. 2010).

Conclusions

The association analysis revealed that the T allele at SNP g.4338T>C (SNP03) improved the heat tolerance (p<0.05) of the animals. Allele T was exclusively found in White Lamphun animals and to 84% in Mountain cattle. Holstein Friesian heifers revealed an allele frequency of only 18%. The study indicates breed specific physiological responses to heat stress. Polymorphisms within *HSP90AB1* were not causative for the physiological responses; however, the results propose that this gene is an attractive candidate for heat tolerance and should at least be used as a genetic marker to select appropriate breeds for hot climates.

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