Journal of Genetics (2020)99:54
https://doi.org/10.1007/s12041-020-01211-z

RESEARCH NOTE

SNP of *AHSA2* gene in three cattle breeds using snapshot technology

SHU-ZHU CHENG[1], E. GUANG-XIN[1], CHENG-LI LIU[1], WANG-DUI BASANG[2], YAN-BIN ZHU[2], RI-SU NA[1], YAN-GUO HAN[1], YAN ZENG[1], XIAO WANG[1], WEI-WEI NI[1], BAI-GAO YANG[1], XING-HAI DUAN[1], ZE-HUI GUO[1], MEIHUA SONG[3] and YONG-FU HUANG[1]*

[1]*College of Animal Science and Technology, Chongqing Key Laboratory of Forage and Herbivore, Chongqing Engineering Research Centre for Herbivores Resource Protection and Utilization, Southwest University, Chongqing 400716, People's Republic of China*
[2]*State Key Laboratory of Barley and Yak Germplasm Resources and Genetic Improvement (Tibet Academy of Agricultural and Animal Husbandry Science (TAAAS)), Lhasa 850002, Tibet, People's Republic of China*
[3]*Qixia Manor Veterinary Station, Qixia, Shandong 265300, People's Republic of China*
*For correspondence. E-mail: H67738337@swu.edu.cn.

Received 27 March 2019; revised 6 March 2020; accepted 12 March 2020

Abstract. Droughtmaster is a tropical breed of beef cattle that can survive in hot climates and easily adapt to torrid environments. These traits are important in livestock breeding. In this study, we genotyped five single-nucleotide polymorphisms (SNPs) of the *AHSA2* gene from 190 cattle belonging to three different breeds (Droughtmaster, Angus and Simmental) by using snapshot technology. This work aimed to identify the valuable molecular marker of heat resistance in cattle. Results showed that Droughtmaster exhibited higher expected heterozygosity and polymorphic information content compared with the two other breeds. The *AHSA2*-1 locus deviated from the Hardy–Weinberg equilibrium in the Droughtmaster breed ($P < 0.05$). Two SNPs in Droughtmaster diverged significantly from Angus and Simmental. The SNPs were identified as *AHSA2*-3 and *AHSA2*-4, which were closely linked to the three breeds based on pair-wise F_{ST}. *AHSA2*-4 involved a missense mutation. In summary, the GG genotypes in *AHSA2*-3 and *AHSA2*-4 may be candidate genotypes associated with heat resistance traits and may serve as valuable genetic markers for breeding of heat-tolerant beef cattle in the future.

Keywords. *AHSA2* gene; heat stress; snapshot technology; single-nucleotide polymorphism.

Introduction

The developments of molecular markers in the late 1980s unfolded new methods for livestock breeding and profoundly affected the entire field of livestock breeding (Strychalski *et al*. 2011; Wang *et al*. 2015). Snapshot is a commercial small-scale sequencing method for medium-throughput SNP typing; it uses fluorescently labelled ddNTPs to amplify SNP adjacent primers (Sachiyo *et al*. 2002; Turner *et al*. 2002; Zhao *et al*. 2003). The snapshot technology can perform accurate typing, which is a convenient and efficient technique for SNP screening, has fast detection speed, has no restriction on SNP site polymorphism and is not limited by the number of samples (Ye *et al*. 2009; Liu 2013; Hu *et al*. 2013).

Heat stress adversely affects the growth and reproduction of animals, causes cell-level responses and changes the expression of related genes (Feige *et al*. 1996; Cai *et al*. 2005). Heat shock proteins (HSPs) are an important family of highly conserved proteins and are also known as stress proteins. When the body receives stress stimulus, it produces nonspecific cells for protection, which play an important role in cell survival and homeostasis (Morimoto 1993; Li and Srivastava 2004; Testori *et al*. 2008). Notably, *AHSA2* is an activator of HSP90 ATPase homolog 2, which has been suggested to act as a general stimulator of Hsp90 function (Sun *et al*. 2012). AHSA2 may play a regulatory role in heat stress responses. Droughtmaster is a tropical breed of beef cattle developed by crossing the Brahman and British cattle breeds in North Queensland (Francis and Little 2010; Sun

Shu-Zhu Cheng, Cheng-Li Liu, E. Guang-Xin contributed equally to this work.

Published online: 09 June 2020

et al. 2017). The breed has good heat tolerance, can take full advantage of its environment and pastures in the most efficient manner for high weight gain and fertility.

In this study, 190 blood samples from three breeds of cattle (63 Droughtmaster, 37 Angus and 90 Simmental) were collected. The genetic diversity and population structure of *AHSA2* and the differences between the three native Chinese cattle breeds were evaluated using five SNPs related to *AHSA2*. Genetic differences among the three breeds were compared. This work provides basic data for cattle breeding in terms of heat tolerance (related to the *AHSA2* gene in native Chinese cattle breeds) and environmental adaptability improvement as well as a theoretical basis for future studies.

Materials and methods

Samples

Samples were collected from 190 cattle of the three breeds in Chongqing, China (table 1). The protocols used for DNA extraction have been previously described by Zhong *et al*. (2011) and E *et al.* (2016).

Initial PCR amplification

Table 2 shows the primers previously described for amplifying SNP-containing regions (Turner *et al*. 2002). The reaction system had a total volume of 10 μL and contained 5 μL of 2× *Taq* PCR Master Mix, 1 μL of primer mix (based on the amplification ratio), 1 μL of DNA template and 3 μL of double-distilled H_2O. The PCR amplification conditions were as follows: predeformation at 95°C for 5 min; 42 cycles of 95°C for 30 s, 56°C for 30 s and 72°C for 30 s and final extension at 72°C for 2 min. The PCR products were purified (Yousef and Hosseini 2019).

Snapshot reactions

The design of the SNaPshot extension primers is previously described by Turner *et al.* (2002). PCR amplification was carried out using 5 μL of the reaction mixture containing 0.5 μL of SNaPshot mix, 3 μL of pooled PCR products, 1 μL of pooled primers and 0.5 μL of double-distilled H_2O. The PCR amplification conditions were as follows: predeformation at 95°C for 2 min; 40 cycles of denaturation at 95°C for 10 s, annealing at 52°C for 5 s and extension at 60°C for 10 s and storage at 4°C. After PCR amplification, the products were purified. We then used a computer for sequencing (Zhang *et al*. 2014; Yu *et al*. 2017).

Data analysis

We used Toolkit software to measure genotype frequency, allele frequency, polymorphism information content (PIC),

Table 1. Sampling information for three cattle breeds.

Name	Code	SZ	N(S)	E	Native	Acquisition site
Angus	A	37	50°N ~ 58°N	2°E ~ 7°W	Scotland	Chongqing
Simmental	X	90	45°N ~ 47°N	5°E ~ 10°E	Switzerland	Beijing
Droughtmaster	K	63	10°S ~ 43°S	112°E ~ 152°E	Australia	Chongqing

SZ, sample size; N, north latitude; S, south latitude; E, east longitude; code is short name of breed.

Table 2. Primer information used in this study and five SNP loci information.

Locus	PCR primers	Single base extension primers	SNP location	FD	SVT	AVT
AHSA2-1	F: TTGCAAGCCATAATGGGAAC R: AGCAAGCCCCAACCCAAAAC	GTAACAGGATCATACATATC TATAAT	Chr11-59776790	Intron	G/A	Synonymous
AHSA2-2	F: AGCCAACTTTTTCACTCTCC R: TATGCAGTTCAGGAAGCAAC	TCTGACTCAAGCTGGAATC AAGATTGCCGG				
AHSA2-3	F: CAACCAAGAATACTACACCC R: CCATTACCAGTGAGCTTTAC	ACTGACTACATCCTTTTATT TCAGCCTGATGA	Chr11-59781761	Intron	G/A	Synonymous
AHSA2-4	F: ACCAACCAAAGCTATGGCAG R: CACAGGCTCTTGAAGCTTTG	GACTGACTGCACGGCATTC TCACTCAGTTTCCTTT	Chr11-59787784	Exon5	G/C	Nonsynonymous
AHSA2-5	F: AGATGCCAGGGTGGATTAAC R: GGAAACTGAGTGAGAATGCC	ACTGACTGACTGATGTGCG TGCGTGCAAGCTCAGTCAT	Chr11-59787901	Intron	G/A	Synonymous

FD, functional domain; SVT, single-nucleotide variation type; AVT, amino acid variation type.

Table 3. Genotype frequency, allele frequency and genetic diversity of four SNP locus of *AHSA2* in three cattle breeds.

Locus		Genotype frequency			Allele frequency		H_O	H_E	PIC	pHWE
AHSA2-1		AA	GA	GG	A	G				
	AS	0	0	1	0	1	0	0	0	
	KH	0.0328	0.0328	0.9344	0.0491	0.9508	0.03279	0.0943	0.8910	0.00306
	XM	0	0	1	0	1	0	0	0	
AHSA2-3		AA	GA	GG	A	G				
	AS	0	0.2432	0.7568	0.1216	0.8784	0.24342	0.21659	0.1908	1.00000
	KH	0.1111	0.3968	0.4920	0.3095	0.6905	0.39683	0.43068	0.33361	0.56286
	XM	0.0112	0.0899	0.8989	0.0562	0.9438	0.08989	0.10665	0.1104	0.23431
AHSA2-4		CG		GG	C	G				
	AS	0.1351		0.8649	0.0676	0.9324	0.13514	0.12773	0.1181	1.00000
	KH	0.3492		0.6508	0.1746	0.8254	0.33333	0.30083	0.2539	0.67141
	XM	0.0899		0.9101	0.0449	0.9551	0.08989	0.08633	0.0822	1.00000
AHSA2-5		GA		GG	A	G				
	AS	0		1	0	1	0	0	0	
	KH	0.0167		0.9833	0.0083	0.9917	0.01667	0.01667	0.0164	1.00000
	XM	0		1	0	1	0	0	0	

H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphic information content; pHWE is number of populations deviated from Hardy–Weinberg equilibrium; *Significance P value (significance level = 0.0500) of variance analysis.

heterozygosity (H_E) and effective number of alleles (Kalinowski *et al*. 2007; Bai *et al.* 2016). We then used GENEPOP 3.4 to detect Hardy–Weinberg balance (Raymond and Rousset 1995; Guan *et al*. 2009). Pairwise differences between the populations (F_{ST}) were assessed with Arlequin software v. 3.5.1.3 (Dhia and Rateau 2005; Excoffier and Lischer 2010). Linkage disequilibrium (LD) pattern within the breeds was visualized using Haploview (Wanjiku *et al*. 2015).

Results

Four SNP loci were screened from the *AHSA2* gene of 190 individuals with polymorphism (table 3), and *AHSA2*-2 were CC genotypes without polymorphism among all individuals. According to the population genetic analysis, the observed genetic diversity (H_O) and expected genetic diversity (H_E) values of the Droughtmaster breed were higher than those of the other breeds in the four SNPs (table 3). In this study, PIC was the highest in the Droughtmaster breed and the lowest in the Angus and Simmental breeds on the basis of the known evolution standard of PIC (Botstein *et al*. 1980). These results indicate that the Droughtmaster breed is rich in genetic diversity compared with the other breeds. In addition, the SNP *AHSA2*-1 ($P < 0.05$) deviated from the HWE *AHSA2* locus in only the Droughtmaster breed. By contrast, no SNPs deviated from HWE in Angus and Simmental.

The genetic divergence between different cattle breeds was measured using the pair-wise differentiation coefficient (F_{ST}). As shown in table 4, F_{ST} estimates at two SNPs of Droughtmaster diverged significantly from Angus and Simmental. However, no SNPs significantly differed between Angus and Simmental. This result is consistent with Nei's genetic distance and the average number of pairwise differences within a population (figure 1).

According to the LD pattern of the *AHSA2* gene from the three breeds, two closely linked SNPs were identified in the three breeds; these SNPs included *AHSA2*-3 and *AHSA2*-4 as well as *AHSA2*-1 and *AHSA2*-3 within Droughtmaster (figure 2). However, the *AHSA2* LD pattern in Droughtmaster was more intense than that in Angus and Simmental (table 5).

Table 4. The pair-wise F_{ST} estimates of two SNPs between three cattle breeds.

SNP	Breed	F_{ST}		
		Droughtmaster	Simmental	Angus
AHSA2-3	Droughtmaster	0.0000		
	Simmental	0.20617*	0.0000	
	Angus	0.08225*	0.02086	0.0000
		Droughtmaster	Simmental	Angus
AHSA2-4	Droughtmaster	0.0000		
	Simmental	0.09201*	0.0000	
	Angus	0.04311*	−0.00438	0.0000

*Significance at $P < 0.05$.

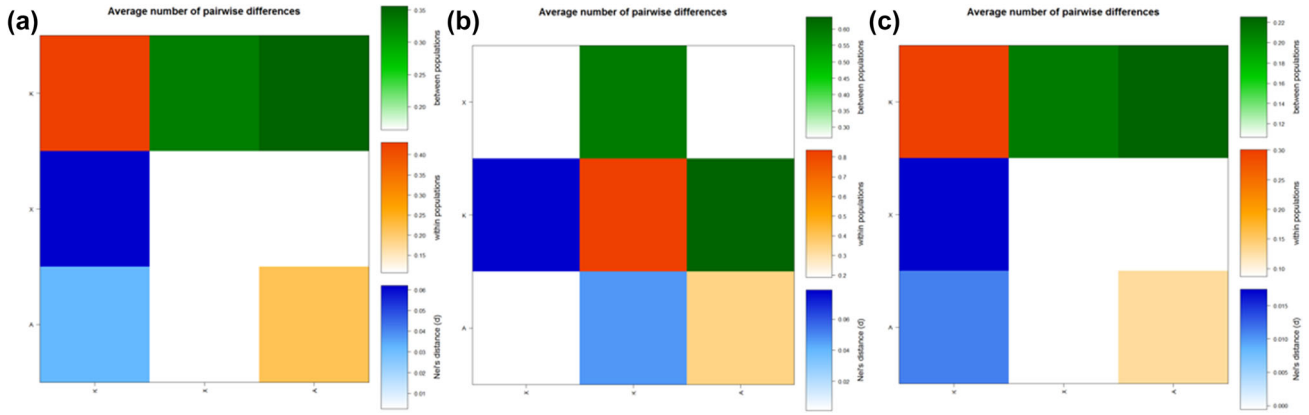

Figure 1. Population average pairwise differences among the three breeds populations. The area above the diagonal shows the average number of pairwise differences between populations, the diagonal elements represent the average number of pairwise differences within population, and the area below the diagonal shows the corrected average pairwise difference (Nei's genetics distance). (a) *AHSA2*-3; (b) *AHSA2*-4; (c) four SNPs.

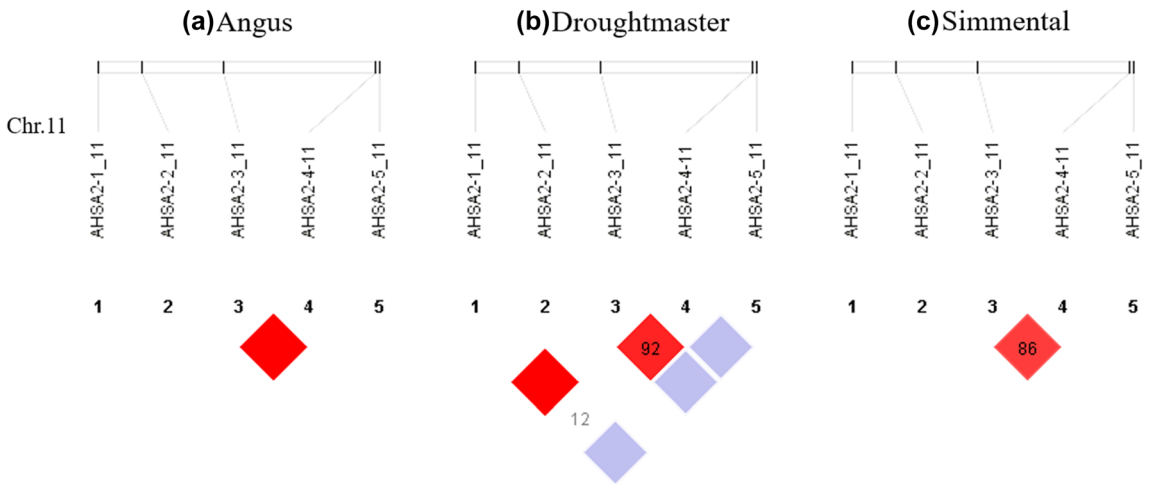

Figure 2. LD in the *AHSA2* gene of three cattle breeds. Each diamond contains a level of LD (D0) between all markerpairs. Darker red tones correspond to increasing levels of D0.

Discussion

In cattle, heat stress is a general term for a series of adverse stress reactions stimulated by high temperature, which can cause decreased food intake, respiratory alkalosis, decreased reproduction and, ultimately, reduced growth performance (Saritvich *et al*. 2016). Heat stress is a key issue in beef cattle breeding (Morton *et al*. 2007; Khan *et al*. 2013; Dash *et al*. 2016). The three different cattle breeds employed in the present study have different breeding histories. Droughtmaster is native to Northern Queensland, Australia. After being introduced to Chongqing, this breed has expressed good environmental adaptability and resistance to parasites, which are very important traits in cattle breeding (Sun *et al*. 2017).

In this work, *AHSA2*-3 and *AHSA2*-4 were closely interlocked in the three populations. The F_{ST} values showed that the Droughtmaster populations showed relatively large genetic difference from the two other breeds. In particular, the LD pattern of the *AHSA2* gene in Droughtmaster was found to have a more intense feature compared with that in the two other breeds, which were not undergoing artificial selection of heat resistance in breeding history. The targeted artificial breeding that results in genome correspondence candidate regulates the frequency of dominant genotypes between generations, thereby forming a tight linkage region (e.g. Toro *et al*. 2019; Li *et al*. 2017).

AHSA2, also known as *AHA1*, is an activator of the heat shock 90-kDa protein ATPase homolog 2 and is also a human gene, which encodes a protein that acts as a

Table 5. LD estimation of *AHSA2* gene within three cattle breeds.

Breeds	L1	L2	D′	LOD	r^2	CIlow	CIhi	Dist	T-int
Droughtmaster	*AHSA2*-1_11	*AHSA2*-3_11	1.0	3.43	0.129	0.52	1.0	4971	3.5
	AHSA2-1_11	*AHSA2*-4-11	0.125	0.07	0.004	0.01	0.57	10994	–
	AHSA2-1_11	*AHSA2*-5_11	1.0	0.0	0.0	0.0	0.0	11111	–
	AHSA2-3_11	*AHSA2*-4-11	0.92	5.95	0.399	0.67	0.98	6023	6.57
	AHSA2-3_11	*AHSA2*-5_11	1.0	0.55	0.021	0.09	0.98	6140	–
	AHSA2-4-11	*AHSA2*-5_11	1.0	0.49	0.042	0.08	0.98	117	1.04
Angus	*AHSA2*-3_11	*AHSA2*-4-11	1.0	3.76	0.523	0.55	1.0	6023	3.76
Simmental	*AHSA2*-3_11	*AHSA2*-4-11	0.865	7.03	0.592	0.58	0.97	6023	7.03

Cilow, the lower bound of 95% of D′ s trusted space; CIhi, the upper bound of D′ s 95% trusted space; LOD, likelihood odds ratio; Dist, the distance (in base) between the loci; T-int, a statistic used by the HapMap Project to measure the completeness of information represented by a set of makers in a region.

cochaperone to Hsp90 (Panaretou *et al*. 2002; Desjardins *et al*. 2012; Shelton *et al*. 2017). *AHSA2* and *AHSA1* are stress-regulating proteins that belong to the AHA family (i.e. activating agent of Hsp90 ATPase); these proteins directly bind to Hsp90 with required Hsp90-dependent client protein activation (Holmes *et al*. 2008). Lotz *et al*. (2003) demonstrated that AHA1 contributes to the efficient activation of the heterologous Hsp90 client protein v-Src. When Hsp90 levels are limited, AHA1 becomes critical for cell viability under nonoptimal growth conditions. Other research also supports this theory (Singh *et al*. 2014; Obermann 2018). Hsp90 is a chaperone protein that aids in the folding of other proteins and stabilises proteins during heat stress; as such, this protein plays an important role in reducing thermal damage and enhancing heat resistance. Therefore, we speculate that the *AHSA2* gene would display an important role in heat stress resistance in cattle.

AHSA2-4 involved a missense mutation. Therefore, the GG genotypes of *AHSA2*-3 and *AHSA2*-4 can be considered as candidate genotypes for studying the heat and parasitic resistance of Droughtmaster. Data from other loci vary widely and may be related to differences in samples among the three breeds.

In conclusion, the results of our gene polymorphism and population genetic analyses indicated that *AHSA2*-3 and *AHSA2*-4 can be used as candidate genes that are related to heat resistance in beef cattle. Our research on the heat resistance traits of the Droughtmaster cattle breed can provide theoretical guidance for future studies.

Acknowledgements

This work was supported by the Characteristic Germplasm Resources Population Selection and Innovation on Mutton Sheep and Goats (no. 2015BAD03B05), National Natural Science Foundation of China (no. 31172195), Fundamental Research Funds for the Central Universities (XDJK2018B014), Chongqing Research Program of Basic Research and Frontier Technology (cstc2018jcyjAX0153), Open Project Program of State Key Laboratory of Barley and Yak Gemplasm Resources and Genetics Improvement (Tibet Academy of Agricultural and Animal Husbandry Sciences (TAAAS)), Lhasa Tibet 850002, China.

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Corresponding editor: H. A. RANGANATH