

Associations Between Cattle Breed and Heat Shock Protein 70 Gene¹

M. Lamb², R. Okimoto³, M. Brown⁴, and C. Rosenkrans, Jr.²

Story in Brief

Heat shock proteins (HSPs) are induced by various stressors such as heat, cold, toxins, and oxygen deprivation. Our objective was to determine the genetic diversity in a segment of the *HSP-70* gene of cattle. Genomic DNA was collected from 157 cows. The cows were *Bos taurus* (Angus; n = 42), *Bos indicus* (Brahman; n = 41), and *Bos taurus/Bos indicus* crosses (n = 74). Specific primers for the bovine *HSP-70* were used for amplification of a 523 base segment using polymerase chain reaction. The amplified gene products were sequenced, and 8 single nucleotide polymorphisms (SNPs) were identified. The SNPs were located at DNA base position 1851 (n = 7; 4.5%), 1899 (n = 1; 0.64%), 1902 (n = 6; 3.8%), 1917 (n = 6; 3.8%), 1926 (n = 6; 3.8%), 2033 (n = 22; 14%), 2087 (n = 10; 6.4%), and 2098 (n = 6; 3.8%). Two SNPs resulted in altered peptide sequences, also known as mis-sense mutations (1926, aspartic acid to glutamic acid, and 2033, glycine to alanine). The occurrence of SNP 2033 was not affected ($P > 0.5$) by breed. Brahman ancestry tended to be related ($P < 0.11$) to the presence of SNPs at positions 1902, 1917, 1926, 2087, and 2098; whereas, SNP 1851 tended to be associated ($P < 0.11$) with Angus. These results indicate that the *HSP-70* gene in cattle is polymorphic, and most of the SNPs identified follow breed lineages.

Introduction

Heat shock proteins (HSPs) are present in all cells of the body but increase in numbers when an animal is subjected to various stressors such as heat, cold, and oxygen deprivation. Heat shock proteins play vital roles in normal cell function by directing other proteins into their right shape, which is essential for function, and then escorting them to the right place at the right time. It has been found in numerous studies that tropically adapted breeds of cattle such as Brahman perform better than some temperate breeds such as Angus in warmer climates. Due to the role of heat shock proteins in the protection of cells against heat stress, and the apparent ability of some breeds to function and perform better than others in heat stress conditions, the possibility of a direct connection between the two is plausible. Therefore, the objective of this study was to determine the genetic diversity of an *HSP-70* gene segment of cattle, and determine if the polymorphisms were associated with breed composition.

Experimental Procedures

Animals. The cows were part of a long-term breeding program at the USDA-ARS Dale Bumpers Small Farms Research Center. Blood samples were collected and the plasma was harvested. Buffy coats were then stored at -112°F to await genomic analysis. Genetic data was successfully collected on 157 cows. The breed composition of the cows and the number of each breed were as follows: *Bos taurus* (Angus; n = 42), *Bos indicus* (Brahman; n = 41), and *Bos taurus/Bos indicus* crosses (n = 74). The crossbred cows were distributed as follows: 38 Angus sired Brahman dams, 36 Brahman sired Angus dams.

Polymerase Chain Reaction (PCR). A Peltier thermal cycler 225 (MJ Research, Waltham, Mass.) was used for amplification. The thermocycler conditions began with a denaturation temperature of 201°F for 2 minutes and then cycled at 201°F for 30 seconds, 131°F for one minute and 154°F for 1 minute. After cycling 35 times, a final extension occurred at 154°F for 10 minutes. Samples were held at 46°F until sequenced.

Primers. Three primers were designed for PCR amplification and sequencing (Sigma-Genosys, Saint Louis, Mo.). Those primers were based on the National Center for Biotechnology Information (NCBI) sequence accession number U09861 of *Bos taurus HSP-70*. Primers HSP1778F (CGCTGGAGTCGTACGCCCTTC) and HSP2326R (CTTGGAAGTAAACAGAAACGGG) were used for amplification of a 548 base pair fragment from positions 1778 to 2326. After amplification, HSP1803F (GAAGAGCGCCGTGGAGGATG) and HSP2326R were used to sequence a 523 base pair fragment within the amplified region from positions 1803 to 2326.

DNA Sequencing. Sequencing was performed by the DNA Core Lab using either the 3100 Genetic Analyzer (Applied Biosystems, Foster City, Calif.) or the CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, Calif.). The primers used for sequencing were the 1803F and 2326R primers. Sequences were analyzed using the DNA Star software.

Statistical Analysis. Breed associations with each single nucleotide polymorphism (SNP) was determined using Chi-square analyses.

Results and Discussion

The bovine *HSP-70* gene was amplified from base 1778 to base 2326. By comparing the region of interest from our samples to the NCBI published sequence (accession number U09861), 8 SNPs

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² Department of Animal Science, Fayetteville

³ Cobb-Vantress, Siloam Springs, Ark.

⁴ USDA-ARS, Grazinglands Research Lab, El Reno, Okla.

were identified with 4 of the 8 resulting in an altered peptide sequence. The SNPs were identified at the following base positions on the *HSP-70* gene: 1851, 1899, 1902, 1917, 1926, 2033, 2087, and 2098.

The frequency and breed composition of each of the 8 SNPs are summarized in Table 1. The base change, location, and effect on the amino acid are summarized in Table 2. The presence of SNP 2033 was not affected ($P > 0.5$) by breed. Brahman ancestry tended to be related ($P < 0.11$) to the occurrence of SNPs at positions 1902, 1917, 1926, 2087, and 2098; whereas, the presence of SNP 1851 tended to be associated ($P < 0.11$) with Angus lineage.

The SNP at position 1851 was found in 7 cows, 4.5% of the total population. Two were Angus purebred and 5 were Brahman-Angus crosses. The base change was from guanine to adenine and displayed the “wobble” effect because it was located in the third position of one of the codons for alanine and resulted in a silent mutation. Only one Brahman/Angus cow, 0.64% of the population, exhibited a SNP at position 1899. Like SNP 1851 the base change was from guanine to adenine in the last base of a codon for leucine and resulted in no amino acid change.

One Angus/Brahman cross, 1 Brahman/Angus cross, and 4 Brahman purebreds, 3.8% of the population, exhibited the same 4 SNP's. A SNP at base 1902 resulted in a base change from cytosine to thymine which occurred in the third position of aspartic acid's codon. A SNP at base 1917 resulted in a guanine to thymine which occurred in the third position of an alanine codon. At base 1926 the SNP resulted in a cytosine to guanine change in the third position of an aspartic acid codon and resulted in an amino acid change to glutamic acid. A base change from thymine to adenine occurred at position 2098. The base change occurred in the first position of the codon would have resulted in an amino acid change of leucine to methionine but occurs after the stop codon.

An SNP at the 2033 position occurred in 22 cows, 14% of the total population, 8 purebred Angus, 5 Angus/Brahman crosses, 3 Brahman/Angus crosses, and 6 purebred Brahman. The base

change occurred in the second codon of glycine, guanine to cytosine, and resulted in an amino acid change to alanine. One animal (3002) was found to be homozygous (CC) for the SNP at this position. This was the only animal and only position found to display a homozygous SNP.

An SNP at 2087 occurred in 10 cows, 6.4% of the population. Two of the animals were Angus/Brahman crosses, 2 were Brahman/Angus crosses, and 6 were purebred Brahman. The base changed is cytosine to guanine. The change takes place at the second position of the codon and would have resulted in a change of serine to cysteine. However, the polymorphism occurred after the stop codon which begins at position 2079.

The SNP's at positions 1902, 1917, 1926, and 2098 appeared to be related. If one was present, they all were present suggesting genetic linkage. That was observed in the genotype of 6 cows; 4 purebred Brahman, 1 Brahman/Angus, and 1 Angus Brahman. The primary objective of this study was to determine the genetic diversity, if any, of the *HSP-70* gene between 2 different species/subspecies of bovine. Our results indicate that the bovine *HSP-70* is polymorphic and breed lineage impacts the occurrence of those genetic differences.

Implications

The national database sequence that these samples were compared to represented the sequence for a *Bos taurus* breed; therefore, the SNPs associated with our *Bos indicus* samples may represent subtle species differences in genetic coding and possibly give rise to future advances in the understanding of the ability of one breed to perform better in heat stress situations than another breed within a genus. Genomic DNA evaluations allow producers to evaluate the genetic potential of animals and, in the future, will increase the accuracy of selecting breeding stock.

Table 1. Effects of breed composition¹ on single nucleotide polymorphisms (SNP) occurrence.

SNP	Sequence position	Frequency ²	Breed			
			AA	AB	BA	BB
1	1851	0.045	2	0	5	0
2	1899	0.006	0	0	1	0
3	1902	0.038	0	1	1	4
4	1917	0.038	0	1	1	4
5	1926	0.038	0	1	1	4
6	2033	0.140	8	5	3	6
7	2087	0.064	0	2	2	6
8	2098	0.038	0	1	1	4

¹ The number of animals with the detected SNP by breed; AA-purebred Angus; AB-Angus sire; BA-Angus dam; BB-purebred Brahman

² Percentage of cows with that SNP in our population of 157 cows

Table 2. Relationship of single nucleotide polymorphisms (SNP) to potential codon position and translational products.

SNP	Base change ¹	Codon position ²	Amino acid change ³
1 (1851)	G to A	3	Ala (no change)
2 (1899)	G to A	3	Leu (no change)
3 (1902)	C to T	3	Asp (no change)
4 (1917)	G to T	3	Ala (no change)
5 (1926)	C to G	3	Asp to Glu
6 (2033)	G to C	2	Gly to Ala
7 (2087)	C to G	-	Post-translational
8 (2098)	T to A	-	Post-translational

¹ G-Guanine; A-Adenine; C-Cytosine; T-Thymine

² 1-first base in codon; 2-second base in codon; 3-third base in codon

³ Ala-alanine; Asp-aspartic acid; Glu-glutamic acid; Gly-glycine; Leu-leucine