GENETIC VARIATION IN BOVINE HEAT SHOCK PROTEIN 40 GENE IN AFRICAN, ASIAN AND AMERICAN CATTLE BREEDS

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Abstract

DnaJ homolog, subfamily A, member 1 (DNAJA1 or Hsp40) encodes a heat shock protein which functions as a co-chaperone of the 70 kDa heat shock protein (Hsp70). Recent work linked it to meat tenderness so we investigated molecular genetic diversity of the N- and C-terminal ends in 15 cattle breeds from Africa, Asia, and North America. We sequenceda total of 1,142 bp of bovine Hsp40 gene representing the N-terminal region (619 bp) and C-terminal region (523 bp) in domestic cattle and compared this to the yak C-terminal region (717 bp). Differences between tropical and temperate breeds possibly suggest genetic differences concomitant with population history and genetic diversity among these breeds. The 124 bp insertion found only in yak DNAJA1 gene represents a remarkable difference of potentially significant functional relevance that warrants further investigation. In-silico functional analysis with PANTHER predicted putative deleterious functional impact of c.161 T>A p.V54Q. Genetic differences in Hsp40 gene between tropical and temperate breeds of cattle may result from evolutionary forces acting during periods of adaptation following domestication, and may have functional consequences for economic traits like heat tolerance and meat tenderness in cattle.

Keywords: Cattle, Hsp40 gene, haplotypes, genetic diversity, Asia, Africa, America

Introduction

Proteins belonging to the 40-kDa heat shock (Hsp40) family protect cells from proteotoxic stress by suppressing protein aggregation and repairing stress-damaged proteins (Harris *etal.*, 2014).The Hsp40 family is large and structurally and functionally diverse and are characterized by the presence of a J-domain which is at the N-terminus in types I and II, a disordered glycine-phenylalanine (G/F)-rich region which is responsible for flexibility and may function as a linker between the J-domain and the C-terminal region of DnaJ proteins (Szyperski *etal.*, 1994).Given their chaperoning function alongside their partner Hsp70s, it was hypothesized that mutations in these regions could directly or indirectly abrogate their chaperoning functions, with attendant consequences on promoting protein misfolding and cellular stress that may result from heat stress. We sequenced and analyzed sequences in the N and C terminal regions of the bovine *DNAJA1* heat shock protein gene in 15 cattle breeds from Asia, Africa and North America as well as yak to better understand molecular footprints in the *DNAJA1* gene that may be characteristic of evolutionary and functional differences in geographically separated breeds of cattle.

Materials and Methods

Genomic DNA from 86 blood samples of 15 cattle breeds and yak were collected from representative samples from Africa (Nigeria), Asia (Pakistan) and USA. Two pairs of PCR primers F: 5' –GTTGAAAGTCGGTTAAAATTGAGC-3' R: 5'-TCATGAAGCCGTGTATACTCCC-3') F: 5' –GGAAGAAAGGGCATCTTTTGAAAT-3' R: 5'-TTGGCACTTCTGGCAATTCAG-3') were designed using NM_001015637.1 as reference sequence to amplify 619 bp spanning exon 2, intron 2 and 3 in the N-terminal region and 523 bp spanning exon 7, intron 7 and exon 8 in the C-terminal region respectively and PCR products were directly sequenced on ABI Automated 3730 DNA Analyzer.Single nucleotide polymorphisms (SNPs) were identified with the aid of CodonCode Aligner software and DnaSP version 5.10.01. Genotypes were obtained from analyzing and comparing the obtained sequence electropherograms. Indices of sequence variation and haplotype structure were calculated using DnaSP version 5.10.01 including number of polymorphic sites and number of haplotypes. *In-silico* functional analysis of missense mutations

was obtained using PANTHER (Thomas *etal.*, 2003) and alignment of the nucleotide sequences was done using ClustalX (Larkin *etal.*, 2007).

Results and Discussion

Two primer pairs were designed to amplify the N-terminal to produce fragments of 619 bp spanning exons 2 and 3 and the C-terminal regions of 523 and 717 base pairs (for domestic cattle and yak respectively) spanning exons 7 and 8 of the bovine DNAJA1 gene (Figure 1). SNPs identified occurred in exons 2 and 3 followed by the presence of invariant sites in intron 2. In the N-terminal region containing the J-domain, alignment of the sequences obtained from the amplified fragments identified 5 polymorphic loci located in exons 2 and 3 while intron 2 remained conserved among cattle ecotypes. This level of conservation of intron 2 observed in a recent study (Marty *et al.*, 2010) may be indicative of its transcriptional role in *DNAJA1* gene expression and splicing. Eleven and 9 haplotypes were identified in Asian and African breeds of cattle with American Brangus (indicine Brahman x taurine Angus) population having two haplotypes. In general, the C-terminal region appears to be largely conserved among African, Asian and American breeds of cattle, similar to yak sequences when aligned together. Notably, a 124 bp insertion in intron 8 was observed in yak and is absent in the cattle *DNAJA1* gene and was not found in the bovine genome database (Figure 1). *In silico* functional analysis using PANTHER predicted moderately high values for non-synonymous mutations. The amino acid altering mutations in the C-terminal region of yak were predicted by PANTHER to be non-deleterious, Deletions and substitutions observed in intron 7 and a 124 bp insertion in intron 8 of the C-terminal region may reflect considerable evolutionary divergence between domestic cattle and yak dating up to 5 Mya (Bradley *et bal.*, 1996; Ritz *et al.*, 2000). Whether the 124 bp insertion observed in yak *DNAJA1* contributes to the stability of *DNAJA1* gene in the yak genome is unknown at the moment, and the functional and/or structural relevance of this insertion awaits further investigation Differences in the C-terminal region SNPs between yak and domestic cattle might be as a result of speciation effects. The missense mutations (p. I310F and p. Y311H) observed in yak was predicted by PANTHER to be non-deleterious which further supported our previous argument that evolutionary avoidance of mutations in the C-terminal region is required for known functional role of binding to client proteins. Kota *et al.* (2009) reported the conserved nature of the C-terminal domain while structure-based mutagenesis studies indicated that a conserved hydrophobic pocket located on the peptide binding fragment of type I Hsp40s works in concert with the conserved zinc-finger-like domain (Genevaux *et al.*, 2002; Summers *et al.*, 2009) to enable the predominantly hydrophobic peptide fragment to be presented to Hsp70.

Conclusion

Genetic differences exists in Hsp40 gene between tropical and temperate cattle from Africa, Asia and America, but the number of mutations observed in American Brangus raised in temperate environment was different from cattle raised in tropical environments. These differences may have been sculpted by evolutionary forces during the course of adaptation of these cattle ecotypes. The conservation of the C-terminal region in domestic cattle and yak suggest its critical role in binding non-native proteins. However, the 124 bp insertion observed in yak may have functional or structural roles that are yet unknown. Taken together, our results support significant diversity at this locus that may have functional impact on heat tolerance and meat tenderness in different cattle breeds.

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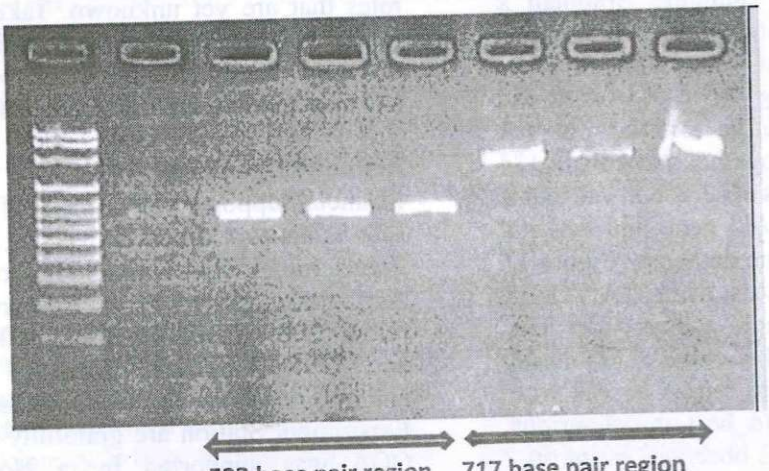

Figure 1: The upper band corresponded to 717 bp r egion observed in yak while the lower band corresponded to 523 bp region of the C –terminal region of the domestic cattle corresponding to peptide binding domain in cattle species.

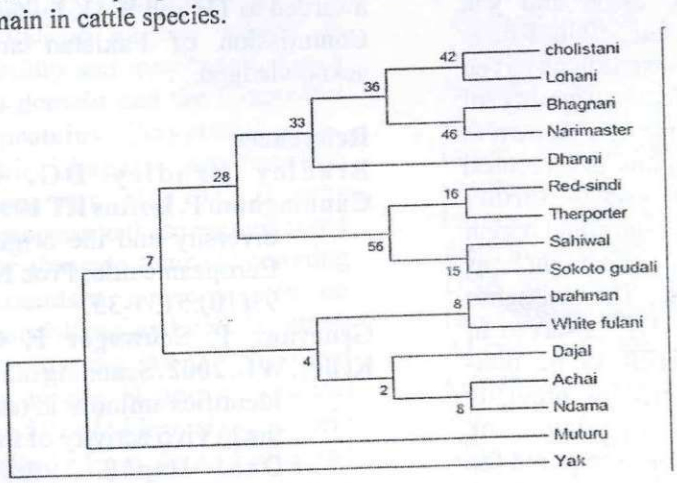

Figure 2: Phylogeny using consensus sequence of Hsp40 gene in yak and 15 cattle breeds.