Indian Journal of Animal Sciences **83** (8): 859–861, August 2013/Short communication

Effect of bovine *DNAJA1* gene polymorphisms on beef tenderness in a commercial crossbred population

K KAPLANOVÁ[1], A DUFEK[2], E DRACKOVA[3], J SIMEONOVOVA[4], J ŠUBRT[5] and I VRTKOVÁ[6]

Mendel University, Zemìdìlská, Brno, Czech Republic

Received: 8 July 2012; Accepted: 3 March 2013

Key words: Beef, *DNAJA1*, Tenderness

The *DNAJA1* (DnaJ homolog, subfamily A, member 1) gene encodes a 40 kDa heat shock protein (Hsp40). The DNAJA1/Hsp70 complex plays a possible role in folding and mitochondrial protein import and directly inhibits apoptosis by preventing mitochondrial translocation of the pro-apoptotic Bax protein (Gotoh *et al.* 2004). Due to anti-apoptotic role, it has been suggested that *DNAJA1* could be involved in meat ageing (Marty *et al.* 2010). Marty *et al.* (2010) have studied genetic variability of *DNAJA1* gene (18 polymorphisms in the 3 main French beef breeds), but no association study evaluating the role of *DNAJA1* gene in tenderness was performed in this day. The present study is focused on *DNAJA1* gene variability and the possible influence of polymorphisms on beef tenderness.

The liver and muscle (*m.quadriceps femoris*) tissues of 4 animals (2 5-years old cows, 2 7-days old calves; Charolais) were used for RNA isolation by RNA Blue (Top-Bio, Prague, Czech Republic), a method based on Chomczynski and Sacchi (1987), purification by DnaseI (New England BioLabs, Beverly. USA) based on Huang *et al.* (1996) and reverse transcription by M-MLV reverse transcriptase. The genomic DNA was isolated from blood and muscle by Blood and Cell DNA Spin Kit and Tissue Spin Kit column-based nucleic acid purification of 12 bulls of 3 cattle breeds (Holstein, Charolais, Czech Fleckvieh), of 60 animals (Czech Fleckvieh, Charolais, Beef Simmental) and of 188 crossbred animals (Czech Fleckvieh × Holstein, Galloway, Beef Simmental, Charolais) In addition the DNA concentration was measured on NanoDrop2000 Spectrophotometer. The primers were designed by Oligo v4.0 (Rychlik and Rhoads 1989). The primer pairs and PCR conditions are listed in Table 1. The PCRs were conducted on thermal cycler with Combi PPP Master Mix in total volume of 15 µl.

The cDNA and genomic DNA PCR products were prepared for sequencing by exonuclease I and alkaline phosphatase, NucleoSEQ Clean-Up of Sequencing and sequenced by Terminator v3.1 Cycle Sequencing Kit. The obtained sequences were compared by Geneious 5.3.4 (Drummond *et al*. 2010) and the new polymorphisms (8) were identified. Two SNPs (g.2583T>G and g.2854C>T) were genotyped by multiplex PCR-RFLP (both the alleles of g.2583 was detected by *Nla*III and alleles of g.2854 by *Hpy*166II), 3 SNPs (g.54C>T, g.329A>G, g.338G>C) by sequencing and SNP in exon 5 (c.621C>T) by allele-specific PCR assay in 60 animals to evaluate allele frequency distribution. Genotyping method, primer pair and PCR conditions are listed in Table 2. The bands were separated in 3% agarose gel for UV visualization.

Alleles and genotypes frequencies were determined according to Hartl and Clark (2007). Further association analysis for statistical evaluation of 3 polymorphisms in 188 crossbred animals was performed. Association analysis with beef quality characteristics (Warner-Bratzler shear force, colour (L, a, b) determined by spektrophotometer, pH by pH-meter texture profile analysis (TPA), intramuscular fat content (%) determined by Soxhlet extraction, water binding capacity by a modification of Grau and Hamm's press method, and cooking loss) was carried out in SPSS 16.0.1 for Windows using GLM Repeated Measures providing analysis of variance for the same measurements that were made several times on each subject – *longissimus* muscle. The beef characteristics were measured during the 2nd, 16th, 30th and 40th day after slaughter. The GLM included aging time as the within-subject factor and effect of sex, diet, sire and genotype as between-subjects factors.

The cDNA obtained from liver and muscle mRNAs of Charolais calves and cows was sequenced and revealed the presence of bovine transcript variant encoded 397 amino acid residues (ENSBTAT00000021637). A fragment of bovine

Present addresses: [1]Ph.D. student (kkaplanova@gmail.com), [5]Scientist (irenav@mendelu.cz), [3]Scientist, Professor (drackova@mendelu.cz, subrt@mendelu.cz), [4]Professor (simeon@mendelu.cz). [2]Research Associate (ales.dufek @vuchs.cz), Agriresearch Rapotín Ltd., Výzkumníkù, Vikýøovice.

Table 1. PCR primers, PCR conditions, amplified and sequenced regions

Primer pair name	Forward;reverse primers (5→3')	Gene regions,amplified fragments (bp)	Ta*
DNAJAE6	AGGTCTCTCTGGGAAAGCTGATG; GCCAATTTCAAGGGAACAGTAAG	Part of intron 5, exon 6, part of intron 6 (1155)	55°C
DNAJAE7	AGCAAGCACAAGGAGGAGGAAG; TTCTAGGGGCTCACAAGAGAGTCA	Part of intron 6, exon 7, part of intron 7 (669)	63°C
DNAJAE8	AACAGAACCATCCTTGAGCTGAG; AGGTAAGGGAAATGGTTGGATGC	Part of intron 7, exon 8, part of intron 8 (636)	60°C
DNAJAE9	TTCTTAAAGCATCCAACCATTTCC; CATACAGGGCAAAGTCAGAGCTT	Part of intron 8, exon 9, part of 3´UTR region (817)	54°C
DNAJA_mRNA1	CGGTGAAAGGCGGAGGAG; TATTAAAACAAAAGCAAGTAGTGAGCATAT	mRNA (1349)	60°C
DNAJA_mRNA2	CGGAGGAGGAGGCAGGA; TATTAAAACAAAAGCAAGTAGTGAGCATAT	mRNA (1018)	60°C

* Ta = annealing temperature.

Table 2. Genotyping protocols of 3 polymorphisms of *DNAJA1* gene

SNP	Primer pair used	Sequence of primer pair	Ta (°C)	PCR product (bp)	Use
g.2854C>T	DNAJAE9_forw	TTCTTAAAGCATCCAACCATTTCC	60°C	747	NlaIII (1.5 U)
g.2583T>G	Dnaja_mRNA_rev	TATTAAAACAAAAgCAAgTagTgAgCATAT		Hpy166II (1.5 U)	
	Dnaja_ex5A	gtggtaaaaaaggagcagtagaatg	67°C	114 (allele T) or	Allele specific assay
c.621C>T	Dnaja_ex5-1B	ctccatgcagacagactgaattta		116 (allele C)	
	Dnaja_ex5-2B	ccatgcagacagactgaatttg			
g.54C>T,		AGGTCTCTCTGGGAAAGCTGATG	55°C	1155	Sequen
g.329A>G	DNAJAE6	GCCAATTTCAAGGGAACAGTAAG			cing
g.338G>C					

Table 3. Allele frequencies for 6 polymorphisms within *DNAJA1* gene in 3 different cattle breeds

Breed	**g.2854T***	**g.2583T**	g.329A	**g.338C**	c.621C	g.54C
Czech Fleckvieh (n = 20)	0.77	0.57	1.00	1.00	0.57	0.95
Charolais(n = 20)	0.63	0.90	1.00	1.00	0.76	0.94
Beef Simmental (n = 20)	0.69	0.74	1.00	1.00	0.65	0.90

*in bold = in association study.

DNAJA1 gene located on BTA8 including the coding exons 6–9 was sequenced in 12 bulls from 3 different cattle breeds. A partial sequence of *DNAJA1* gene was deposited into NCBI database, accession number JN656714.

Five single nucleotide polymorphisms were identified: 1 SNP in intron 5 (JN656714: g.54C>T), 3 SNPs in intron 8 (previously reported in SNP database of NCBI: rs135077905 (g.2583T>G), g.2409G>T and g.2596C>T) and 1 SNP in 3´untranslated region (g.2854C>T). The cDNA sequencing revealed 3 SNPs – 2 nonsynonymous SNPs in exon 6 (JN656714: g.329A>G (Ile > Val) and g.338G>C (Asp > His)), 1 SNP in exon 5 caused the stop codon presence (NM_001015637: c.621C>T). Total 6 SNPs (g.2583T>G, g.2854C>T, g.54C>T, g.329A>G, g.338G>C, c.621C>T were genotyped in 60 animals of beef and dual-purpose breeds in the Czech Republic to evaluate allele frequency (Table 3). Further 3 polymorphic SNPs (g.2854, g.2583 and c.621) were genotyped in 188 crossbred animals to evaluate the association between *DNAJA1* polymorphisms and beef tenderness.

The various authors in their studies show that the gene explained 63% of the tenderness variability, because a strong correlation between *DNAJA1* expression level and tenderness confirmed using a gene expression analysis approach in Charolais muscle samples (Bernard *et al.* 2007, Marty *et al.* 2010). However in our commercial crossbred population only association between SNP in 3´untranslated region (UTR; g.2854) and water binding capacity was found (*CC* > *CT* > *TT;* $P \leq 0.05$*)* and between SNP in intron 8 (g.2583) and TPA was observed (*GT* > *TT*, $P \leq 0.01$), but it revealed no

significant association between missense mutation in exon 5. Our results indicated that polymorphisms identified in this study did not influence beef tenderness and the SNPs are not important source of variability for this trait in our cattle population. Just SNP in intron 8 could be more informative and the effect should be verified in different population. Further analysis should be also focused on variability analysis of non-coding regions, because if expression level correlates with tenderness, the special attention should be paid to promoter region which could affect the amount of expressed *DNAJA1* and consequently tenderness.

SUMMARY

The *DNAJA1* gene encodes a member of the large 40 kDa heat shock protein family (Hsp40). Sequencing of partial genomic DNA (introns 6–8, exons 6–9, part of 3´UTR region; JN656714) and cDNA revealed 8 polymorphisms. Association analysis (n = 188) between g.2583 (intron 8), g.2854 (3´UTR) a c.621 (exon 5) and beef tenderness revealed significant effects of the SNPs only on water binding capacity and texture profile analysis. The results indicated polymorphisms did not affect beef tenderness. The effect of SNP in intron 8 (association with texture profile) should be verified in different population.

ACKNOWLEDGEMENT

This study was supported by Ministry of Education, Youth and Sports of the Czech Republic, projects no. 2B08037 and MSM2678846201, Ministry of Agriculture of the Czech Republic projects no. QI91A055.

REFERENCES

Bernard C, Cassar-Malek I, Le Cunff M, Dubroeucq H, Renand G and Hocquette J-F. 2007. New indicators of beef sensory quality revealed by expression of specificgenes. *Journal of Agricultural and Food Chemistry* **55**: 5229–37.

Chomczynski P and Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry* **162**: 156–59.

Gotoh T, Terada K, Oyadomari S and Mori M. 2004. Hsp70–DnaJ chaperone pair prevents nitric oxide- and CHOP-induced apoptosis by inhibiting translocation of Bax to mitochondria. *Cell Death and Differentiation* **11**: 390–402.

Hartl D L and Clark A G. 2007. *Principles of Population Genetics*. Pp 652. 4th edn. Sinauer Associates, Sunderland, Massachusetts, USA.

Huang Z, Fasco M J and Kaminsky L S. 1996. Optimization of DNase I removal of contaminating DNA from RNA for use in quantitative RNA-PCR. *BioTechniques* **20**: 1012–20.

Marty A, Amigues Y, Servin B, Renand Y, Leveziel H and Rocha D. 2010. Genetic variability and linkage disequilibrium patterns in the bovine *DNAJA1* gene. *Molecular Biotechnology* **44**: 190–97.

Rychlik W and Rhoads R E. 1989. A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and *in vitro* amplification of DNA. *Nucleic Acids Research* **17**: 8543–51.